Supplementary Information

Methyl Scanning Approach for Enhancing the Biological Activity of the Linear Peptidic Natural Product, Efrapeptin C

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64 pages

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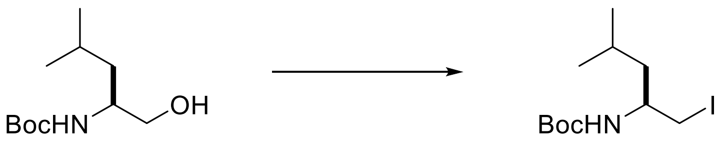
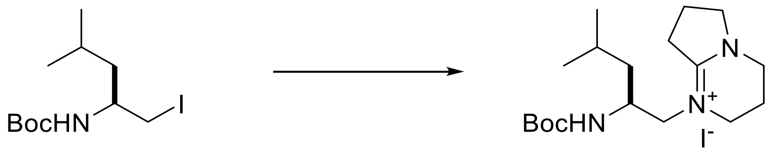
Methods

General Remarks. All reactions sensitive to air or moisture were carried out under argon (Ar) atmosphere in dry solvents unless otherwise noted. CH_2Cl_2, DMF, and Et_2O were purified by a Glass Contour solvent dispensing system (Nikko Hansen). All other reagents were used as supplied unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using Merck Silica gel 60 F_{254} pre-coated plates (0.25 mm). Flash column chromatography was performed using 40–50 μm Silica Gel 60N (Kanto Chemical). Solid-phase peptide synthesis (SPPS) was performed on a microwave-assisted peptide synthesizer MWS-1000 (EYELA) using a sealed reaction vessel, the reaction temperature of which was monitored by an internal temperature probe, or an automated peptide synthesizer Initiator + Alstra (Biotage). Melting points were measured on Yanaco MP-J3 micro melting point apparatus, and were uncorrected. Optical rotations were measured on a P-2200 polarimeter (JASCO) at room temperature using sodium D line. Infrared (IR) spectra were recorded on FT/IR-4100 spectrometer (JASCO) as a thin film on KBr or CaF_2. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a JNM-ECS-400 (400 MHz for ^{1}H NMR, 100 MHz for $^{13}C\{^{1}H\}$ NMR) spectrometer (JEOL), or a JNM-ECX-500 (500 MHz for ^{1}H NMR, 125 MHz for $^{13}C\{^{1}H\}$ NMR) spectrometer (JEOL). Chemical shifts were reported in ppm on δ scale relative to $CHCl_3$ (δ 7.26 for ^{1}H NMR), $CDCl_3$ (δ 77.0 for $^{13}C\{^{1}H\}$ NMR), DMSO-d_5 (δ 2.50 for ^{1}H NMR), DMSO-d_6 (δ 39.5 for $^{13}C\{^{1}H\}$ NMR), CD_2HOD (δ 3.31 for ^{1}H NMR), and CD_3OD (δ 49.0 for $^{13}C\{^{1}H\}$ NMR) as internal references. Signal patterns are indicated as s, singlet; d, doublet; dd, double doublet; m, multiplet peak. High-resolution mass (HRMS) spectra were recorded on a T100LP (JEOL) or a micrOTOF II (Bruker Daltonics) electrospray ionization time-of-flight (ESI-TOF) mass spectrometer. MALDI-TOF MS/MS analysis were performed on a TOF/TOF 5800 system (AB Sciex). UV absorbance was measured on a UV-1800 UV-VIS spectrophotometer (Shimadzu). High-performance liquid chromatography (HPLC) experiments were performed on a HPLC system equipped with a PU-4180 RHPLC pump (JASCO) or a HPLC system equipped with a PU-2089 Plus intelligent pump (JASCO). Ultrahigh-performance liquid chromatography (UHPLC) experiments were performed with an Extrema system (JASCO).



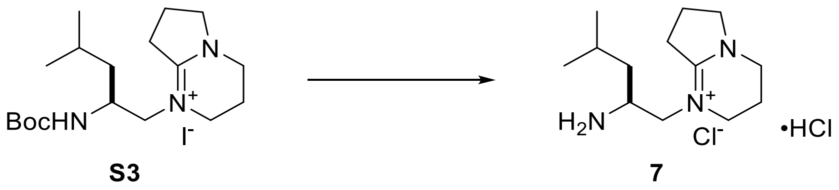
S1 **S2**

Iodide S2 [CAS 161529-19-7].[S1] Boc-protected L-leucinol **S1** [CAS 82010-31-9] was prepared according to the literature.[S2] To a solution of PPh_3 (2.90 g, 11.0 mmol) in CH_2Cl_2 (24.0 mL) were added I_2 (1.87 g, 7.36 mmol) and imidazole (1.25 g, 18.4 mmol) at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was added to a solution of **S1** (800 mg, 3.68 mmol) in CH_2Cl_2 (8.00 mL). The reaction mixture was stirred at room temperature for 3 h. The resultant mixture was filtered, washed with brine (20 mL × 3), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (150 g, *n*-hexane/EtOAc 100/1 to 20/1) to give **S2** (719 mg, 2.20 mmol, 60%): colorless solid. IR (film) 3332, 2959, 1693, 1505, 1366, 1252, 1168, 1011 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.51 (1H, br s), 3.46–3.27 (3H, m), 1.63 (1H, m), 1.45 (9H, s), 1.34 (2H, dd, *J* = 7.3, 6.8 Hz), 0.94 (3H, d, *J* = 6.4 Hz), 0.93 (3H, d, *J* = 6.4 Hz). HRMS (ESI-TOF) m/z: Calcd for $C_{11}H_{22}INO_2Na$ $[M+Na]^+$ 350.0587; Found 350.0602. The $[\alpha]_D$, ^{1}H and $^{13}C\{^1H\}$ NMR spectra of **S2** were identical to those reported previously.

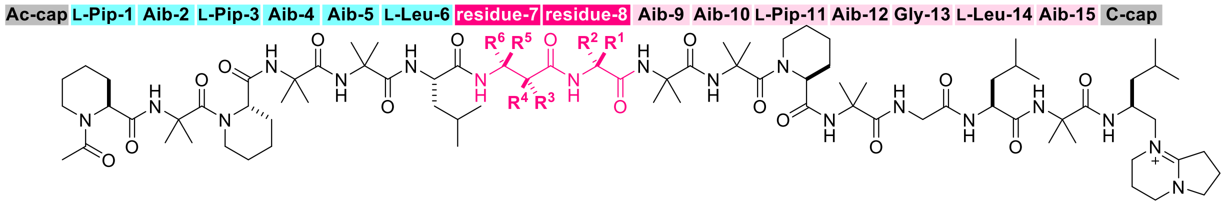


S2 **S3**

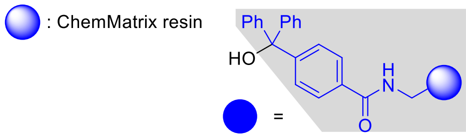
Boc-protected C-cap S3. A solution of iodide **S2** (33.4 mg, 102 μmol) and 1,5-diazabicyclo[4.3.0]non-5-ene (25.2 μL, 204 μmol) in toluene (783 μL) was heated to reflux and stirred for 2 h. The reaction mixture was concentrated. The residue was purified by flash column chromatography on silica gel (2.16 g, $CHCl_3$/MeOH 20/1 to 10/1) to give **S3** (33.3 mg, 73.8 μmol, 72%): yellow foam. $[\alpha]_D^{22}$ +8.32 (*c* 0.64, $CHCl_3$). IR (film) 3257, 2956, 2871, 1699, 1664, 1515, 1452, 1389, 1365, 1312, 1253, 1167 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (1H, d, *J* = 7.8 Hz), 3.93–3.87 (2H, m), 3.76–3.53 (5H, m), 3.43–3.31 (4H, m), 2.35–2.18 (4H, m), 1.73–1.58 (2H, m), 1.41 (9H, s), 1.28–1.25 (1H, m), 0.95 (3H, d, *J* = 6.8 Hz), 0.92 (3H, d, *J* = 6.4 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) 165.3, 155.9, 79.4, 58.5, 54.5, 46.9, 45.7, 42.5, 40.3, 32.2, 28.2 (3 C), 24.7, 22.9, 21.6, 18.9, 18.4. HRMS (ESI-TOF) m/z: Calcd for $C_{18}H_{34}N_3O_2^+$ $[M]^+$ 324.2646; Found 324.2642.



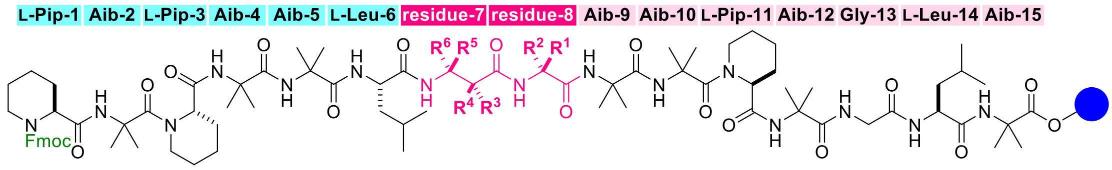
C-cap hydrochloride 7. To **S3** (698 mg, 1.55 mmol) was added 6 M aqueous HCl/MeOH (3/2, 15.5 mL) at room temperature. After being stirred at 30 °C for 1.5 h, the reaction mixture was concentrated. The residue was azeotropically dried with toluene to give **7** (443 mg, 1.50 mmol, 97%): yellow solid. m.p. 246–248 °C. $[\alpha]_D^{20}$ +4.32 (*c* 0.17, $CHCl_3$). IR (film) 3414, 2954, 2871, 2053, 1664, 1535, 1454, 1389, 1312, 1033 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 3.84–3.79 (2H, m), 3.71–3.58 (4H, m), 3.51–3.44 (3H, m), 3.13–3.07 (2H, m), 2.25–2.21 (4H, m), 1.83–1.77 (1H, m), 1.57–1.52 (2H, m), 1.03 (3H, d, *J* = 6.4 Hz), 1.02 (3H, d, *J* = 6.0 Hz). $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) 167.3, 56.2, 56.0, 48.4, 46.2, 43.6, 40.5, 32.7, 25.4, 22.9, 22.5, 19.7, 19.0. HRMS (ESI-TOF) m/z: Calcd for $C_{13}H_{26}N_3^+$ $[M]^+$ 224.2121; Found 224.2118.



compounds	R[1]	R[2]	R[3]	R[4]	R[5]	R[6]
1a	H	H	H	H	H	H
1b	Me	H	H	H	H	H
1c	H	Me	H	H	H	H
1d	H	H	Me	H	H	H
1e	H	H	H	Me	H	H
1f	H	H	H	H	Me	H
1g	H	H	H	H	H	Me

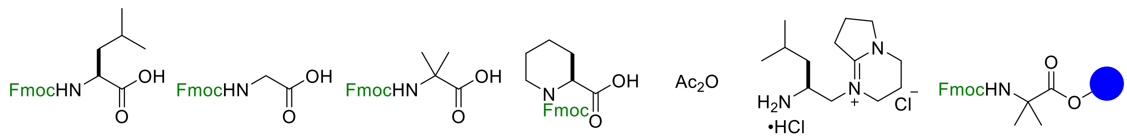


S4 (trityl-OH-ChemMatrix)



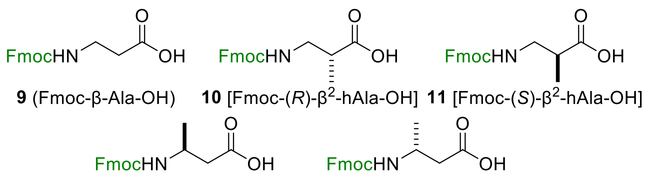
compounds	R[1]	R[2]	R[3]	R[4]	R[5]	R[6]
S5a	H	H	H	H	H	H
S5b	Me	H	H	H	H	H
S5c	H	Me	H	H	H	H
S5d	H	H	Me	H	H	H
S5e	H	H	H	Me	H	H
S5f	H	H	H	H	Me	H
S5g	H	H	H	H	H	Me

common components



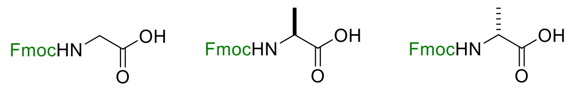
2 **3** **4** **5** **6** **7** **8**

components for residue-7



9 (Fmoc-β-Ala-OH) **10** [Fmoc-(*R*)-β^2-hAla-OH] **11** [Fmoc-(*S*)-β^2-hAla-OH] **12** [Fmoc-(*S*)-β^3-hAla-OH] **13** [Fmoc-(*R*)-β^3-hAla-OH]

components for residue-8



3 (Fmoc-Gly-OH) **14** (Fmoc-L-Ala-OH) **15** (Fmoc-D-Ala-OH)

Figure S1. Structures of **1a–1g**, **S4**, **S5a–S5g**, and synthetic components **2–15** for solid-phase peptide synthesis (SPPS) of **1a–1g**.

Preloaded resin 8. To trityl-OH-ChemMatrix resin (**S4**, 311 mg, 186 μmol) in 20 mL LibraTube (Hipep Laboratories) was added $SOCl_2/CH_2Cl_2$ (1/51, 2.75 mL) at room temperature. After being stirred at room temperature for 12 h, the reaction mixture was filtered. The same procedure was repeated. The resultant resin was washed with CH_2Cl_2 (4.00 mL × 5) and *N*-methylmorpholine/CH_2Cl_2 (1/49, 4.00 mL × 5). To the above resin were added a solution of **4** (364 mg, 1.12 mmol) in CH_2Cl_2 (1.42 mL) and a solution of *N*-methylmorpholine (164 μL, 1.49 mmol) at room temperature. After being stirred at room temperature for 12 h, the reaction mixture was filtered. The same procedure was repeated. To the resultant resin was added *N*-methylmorpholine/MeOH (1/3, 2.00 ml) at room temperature. After being stirred at room temperature for 1 h, the resultant mixture was filtered, and washed with CH_2Cl_2 (4.00 mL × 5).

To the above resin was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 2.00 mL) at room temperature for capping the remaining hydroxy groups. After being stirred at room temperature for 15 min, the reaction mixture was filtered, washed with CH_2Cl_2 (4.00 mL, 40 sec × 5), NMP (4.00 mL, 40 sec × 5), MeOH (4.00 mL, 40 sec × 5), and Et_2O (4.00 mL, 40 sec × 5), and dried under vacuum to give preloaded resin **8** (336 mg).

Determination of loading rate. Fmoc-protected resin **8** was treated with piperidine/NMP (1/1, 400 μL) at room temperature for 30 min and the supernatant was collected. The supernatant (60.0 μL) was diluted with NMP (2.94 mL). UV absorption at 301 nm of the resultant solution was measured. The background absorbance was canceled by subtracting the control absorbance obtained from a solution of piperidine/NMP (1/99, 3.00 mL). The loading rate (x mmol/g) was determined by the following equation S1, where a is the weight of Fmoc-protected resin (mg), and b is absorbance at 301 nm.

$$x = (20000 \times b) / (7800 \times a) \qquad \text{(S1)}$$

Procedures for solid-phase peptide synthesis (SPPS). The resin-bound peptides **23a–23g** were prepared on a peptide synthesizer MWS-1000 (EYELA) or Initiator + Alstra (Biotage).

Standard operation A for conjugating **2** for residues-14 and -6, **3** for residue-13, **4** for residues-12 and -5, **5** for residues-11, -3, and -1, **9**/**10**/**11**/**12**/**13** for residue-8, **3**/**14**/**15** for residue-7 was shown as follows:

Step 1: The solid supported N_α-Fmoc peptide was deprotected with piperidine/NMP (1/4, 40 °C, 5 min × 2).

Step 2: The resin in a reaction vessel (5 or 20 mL LibraTube) was washed with NMP (2.00 mL for 5 mL LibraTube, 4.00 mL for 20 mL LibraTube, 40 sec × 5).

Step 3: An N_α-Fmoc-protected amino acid (4.0 eq) in a vial was activated by a solution of *O*-(7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 4.0 eq, 0.45 M)/1-hydroxy-7-azabenzotriazole (HOAt, 4.0 eq, 0.45 M) in NMP. To the solution of activated amino acid was added a solution of *i*-Pr_2NEt (8.0 eq, 2.0 M) in NMP. The resultant mixture was transferred to the reaction vessel.

Step 4: The activated N_α-Fmoc-protected amino acid was coupled with the peptide on the resin (60 °C, 20 min), and the reaction vessel containing the resin was washed with NMP (2.00 mL for 5 mL LibraTube, 4.00 mL for 20 mL LibraTube, 40 sec × 5).

Steps 1−4 were repeated and amino acids were condensed on the solid support. For residues-12 and -5, Steps 3 and 4 were repeated (×2). Steps 2 and 4 were carried out under a stream of N_2. Steps 1 and 3 were carried out under atmosphere of N_2.

Standard operation B for conjugating **4** for residues-10, -9, -4, and -2 was shown as follows:

Step 1: The solid supported N_α-Fmoc peptide was deprotected with piperidine/NMP (1/4, 40 °C, 5 min × 2).

Step 2: The resin in a reaction vessel (5 or 20 mL LibraTube) was washed with NMP (2.00 mL for 5 mL LibraTube, 4.00 mL for 20 mL LibraTube, 40 sec × 5).

Step 3: N_α-Fmoc-protected Aib **4** (4.0 eq) in a vial was activated by a solution of 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU, 4.0 eq, 0.4 M) in NMP. To the solution of activated amino acid was added a solution of *i*-Pr_2NEt (8.0 eq). The resultant mixture was transferred to the reaction vessel.

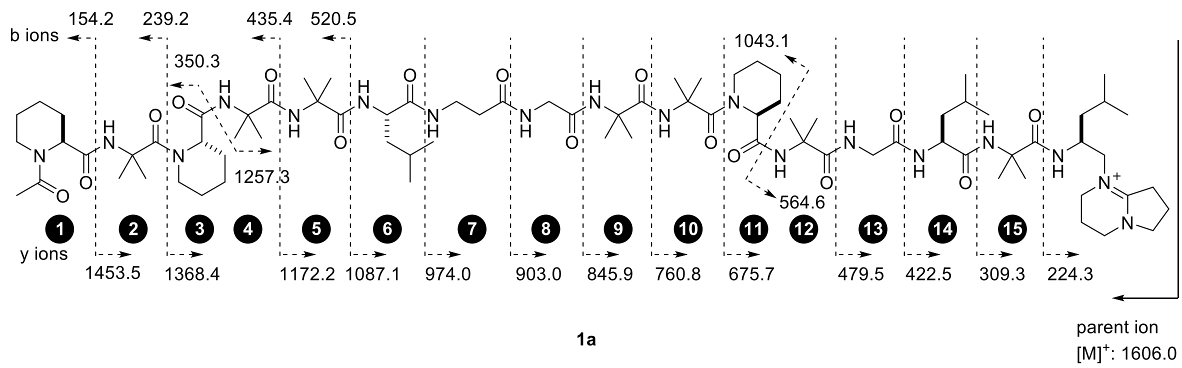
Step 4: The activated N_α-Fmoc-protected amino acid was coupled with the peptide on the resin (40 °C, 2 h), and the reaction vessel containing the resin was washed with NMP (2.00 mL for 5 mL LibraTube, 4.00 mL for 20 mL LibraTube, 40 sec × 5).

Steps 1−4 were repeated and amino acids were condensed on the solid support. Steps 3 and 4 were repeated (×2). Steps 2 and 4 were carried out under a stream of N_2. Steps 1 and 3 were carried out under atmosphere of N_2.

Synthesis of 1a. The preloaded resin **8** (79.9 mg, 20.5 μmol, loading rate: 0.256 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 5 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.
The above resin-bound peptide **18** was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **9** for residue-7) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S5a**.
The above 15-mer N_α-Fmoc-protected resin-bound peptide in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine.
To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23a** (93.9 mg).
To **23a** (25.0 mg, 5.45 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24a**. The crude **24a** was dissolved into *i*-PrOH and filtered through a PTFE filter with 0.20 μm of pore size in

the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil C8-3 10 × 250 mm, eluent A: *i*-PrOH, eluent B: H_2O, linear gradient A/B = 70/30 to 100/0 over 30 min, flow rate: 1.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **24a** (t_R = 29.2–47.6 min, 2.65 mg), which was used for the next reaction without further purification.

To a solution of **7** (7.29 mg, 24.6 μmol) in DMF (50.0 μL) was added Et_3N (3.43 μL, 24.6 μmol). After removing $Et_3N{\cdot}HCl$ by centrifugation at 112 × *g* for 5 min at room temperature, the corresponding free amine was obtained from the supernatant. To a solution of **24a** (1.15 mg, 0.820 μmol) in DMF (151 μL) in 1 mL sealed tube were added a solution of COMU (7.03 mg, 16.4 μmol) in DMF (20.0 μL), a solution of the above free amine (24.6 μmol) in DMF (55.0 μL), and *i*-Pr_2NEt (4.29 μL, 24.6 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 1.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil C8-3 4.6 × 150 mm, eluent A: *i*-PrOH, eluent B: H_2O, linear gradient A/B = 90/10 to 100/0 over 60 min, flow rate: 0.500 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1a** (t_R = 16.3–20.1 min, 978 μg, 26% over 32 steps): colorless foam. $[\alpha]_D^{28}$ +14.7 (*c* 0.031, MeOH). IR (film) 3743, 3673, 3649, 3304, 2944, 2376, 2312, 1652, 1538, 1455, 1203 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): see Table S4. $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6): see Table S5. HRMS (ESI-TOF) m/z: Calcd for $C_{80}H_{137}N_{18}O_{16}^+$ $[M]^+$ 1606.0454; Found 1606.0438.



1a

Figure S2. MS/MS fragmentation pattern of **1a**.

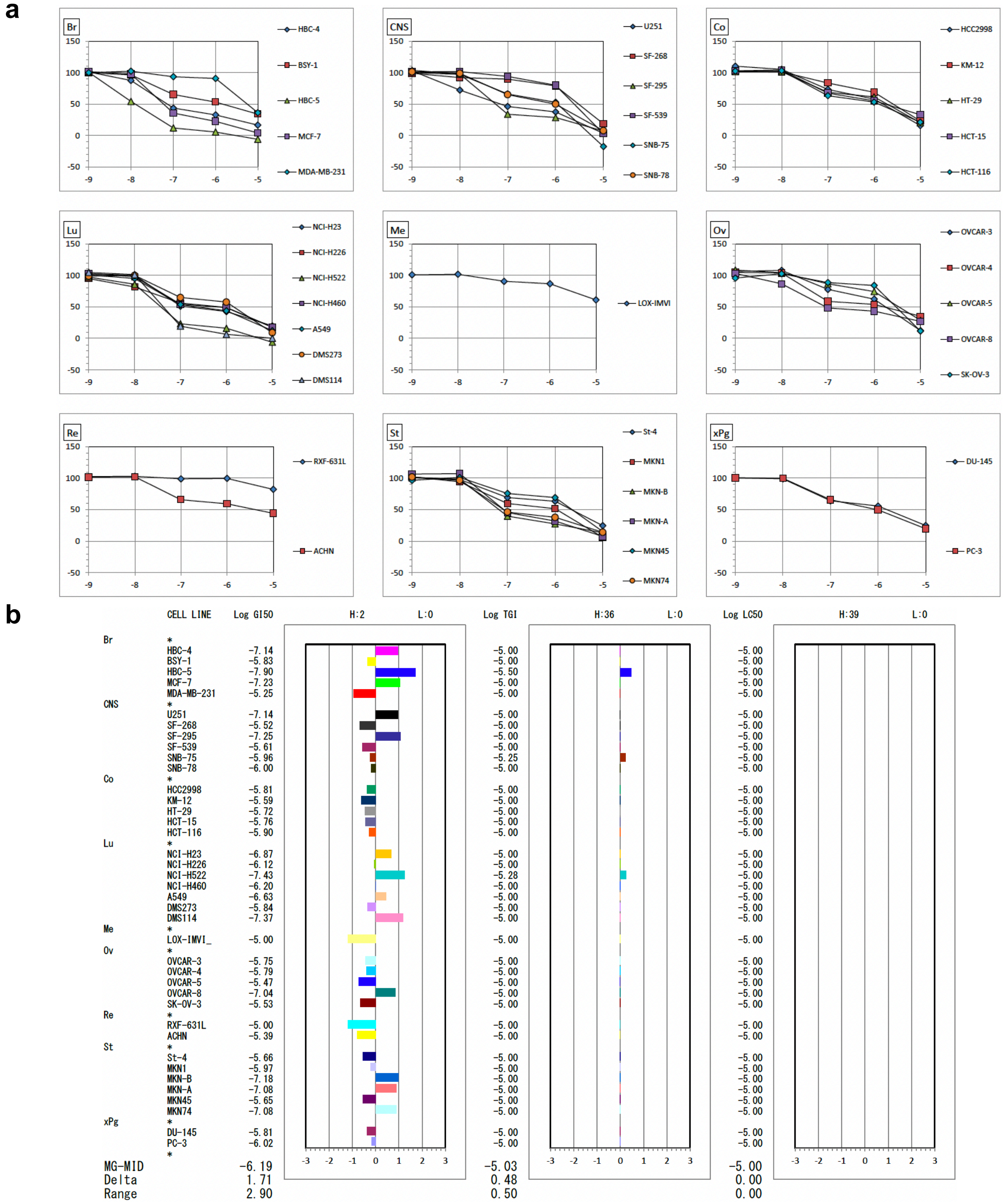


Figure S3. JFCR39 cancer cell panel assay of efrapeptin C (**1a**). (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1a**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1a**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 646 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

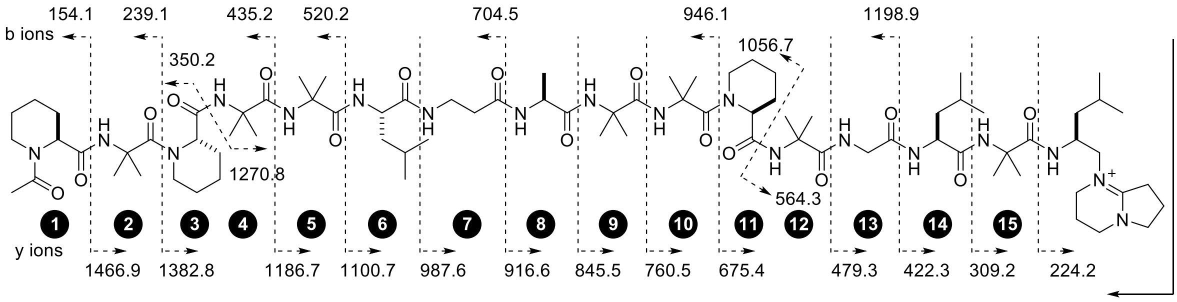
Synthesis of 1b. The preloaded resin **8** (333 mg, 129 μmol, loading rate: 0.386 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

The above resin-bound peptide **18** (57.1 mg, 19.3 μmol) was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **9** for residue-7, **14** for residue-8) to give the 15-mer N_{α}-Fmoc-protected resin-bound peptide **S5b**.

The resin-bound peptide **S5b** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine. To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23b**.

To **23b** (14.6 mg, 6.65 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24b**, which was used for the next reaction without further purification.

To a solution of **24b** (6.65 μmol) in DMF (1.00 mL) in 5 mL sealed tube were added a solution of COMU (56.9 mg, 133 μmol) in DMF (862 μL), **7** (59.1 mg, 199 μmol), and *i*-Pr_2NEt (69.5 μL, 399 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1b** (t_R = 41.0–43.9 min, 605 μg, 5.2% over 32 steps): colorless foam. $[\alpha]_D^{24}$ +15.7 (*c* 0.049, MeOH). IR (film) 3419, 2362, 1680, 1444, 1205, 1140 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0611.



1b

parent ion
$[M]^+$: 1620.0

Figure S4. MS/MS fragmentation pattern of **1b**.

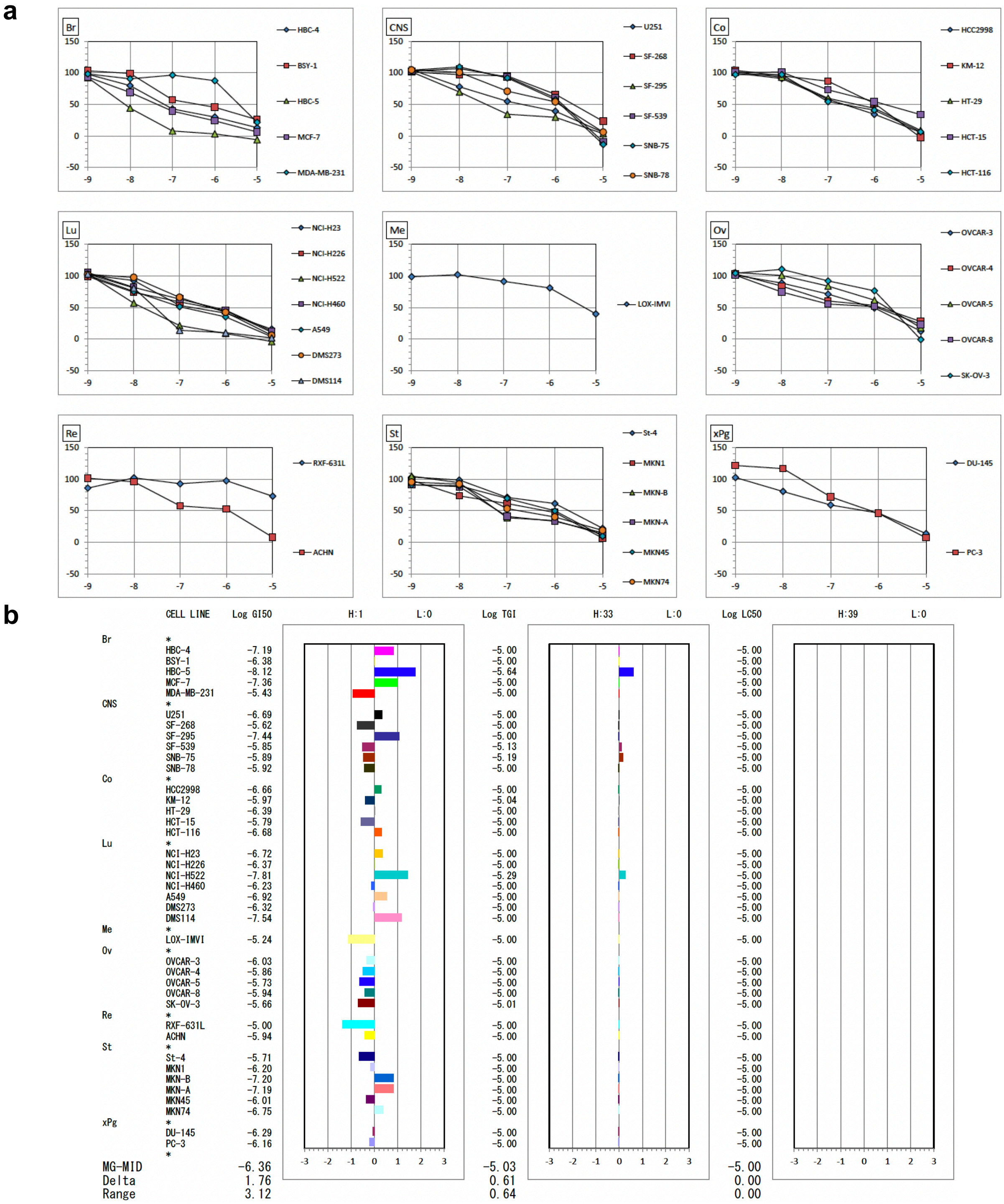


Figure S5. JFCR39 cancer cell panel assay of **1b**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1b**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1b**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 437 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

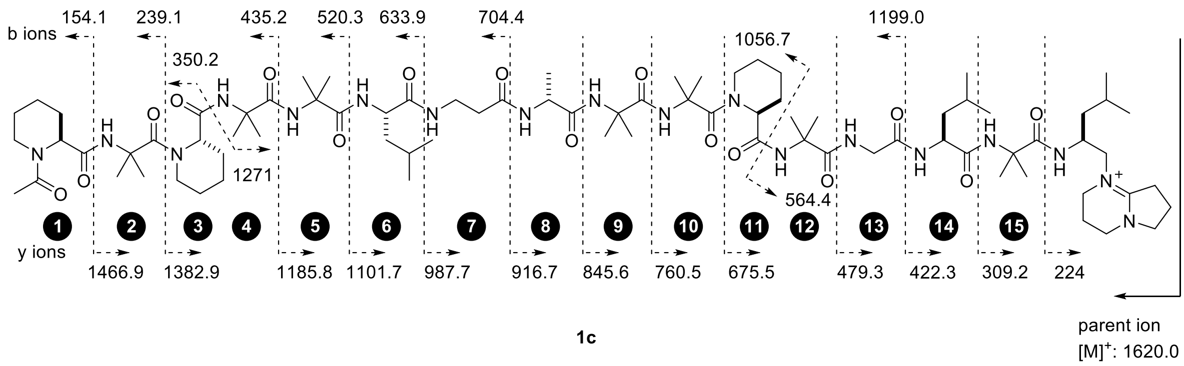
Synthesis of 1c. The preloaded resin **8** (333 mg, 129 μmol, loading rate: 0.386 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

The above resin-bound peptide **18** (58.5 mg, 19.7 μmol) was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **9** for residue-7, **15** for residue-8) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S5c**.

The resin-bound peptide **S5c** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine. To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23c**.

To **23c** (27.8 mg, 11.3 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24c**, which was used for the next reaction without further purification.

To a solution of **24c** (11.3 μmol) in DMF (2.00 mL) in 5 mL sealed tube were added a solution of COMU (96.9 mg, 226 μmol) in DMF (1.17 mL), **7** (101 mg, 339 μmol), and *i*-Pr_2NEt (118 μL, 679 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give two separate peaks, peak 1 (t_R1 =25.9–27.6 min) and peak 2 (t_R2 =29.7–31.8 min), then the peaks were combined to give **1c** (1.84 mg, 9.4% over 32 steps): colorless foam. $[\alpha]_D^{26}$ +34.6 (*c* 0.057 MeOH). IR (film) 3303, 2359, 1678, 1443, 1208, 1139 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0631.



1c

Figure S6. MS/MS fragmentation pattern of **1c**.

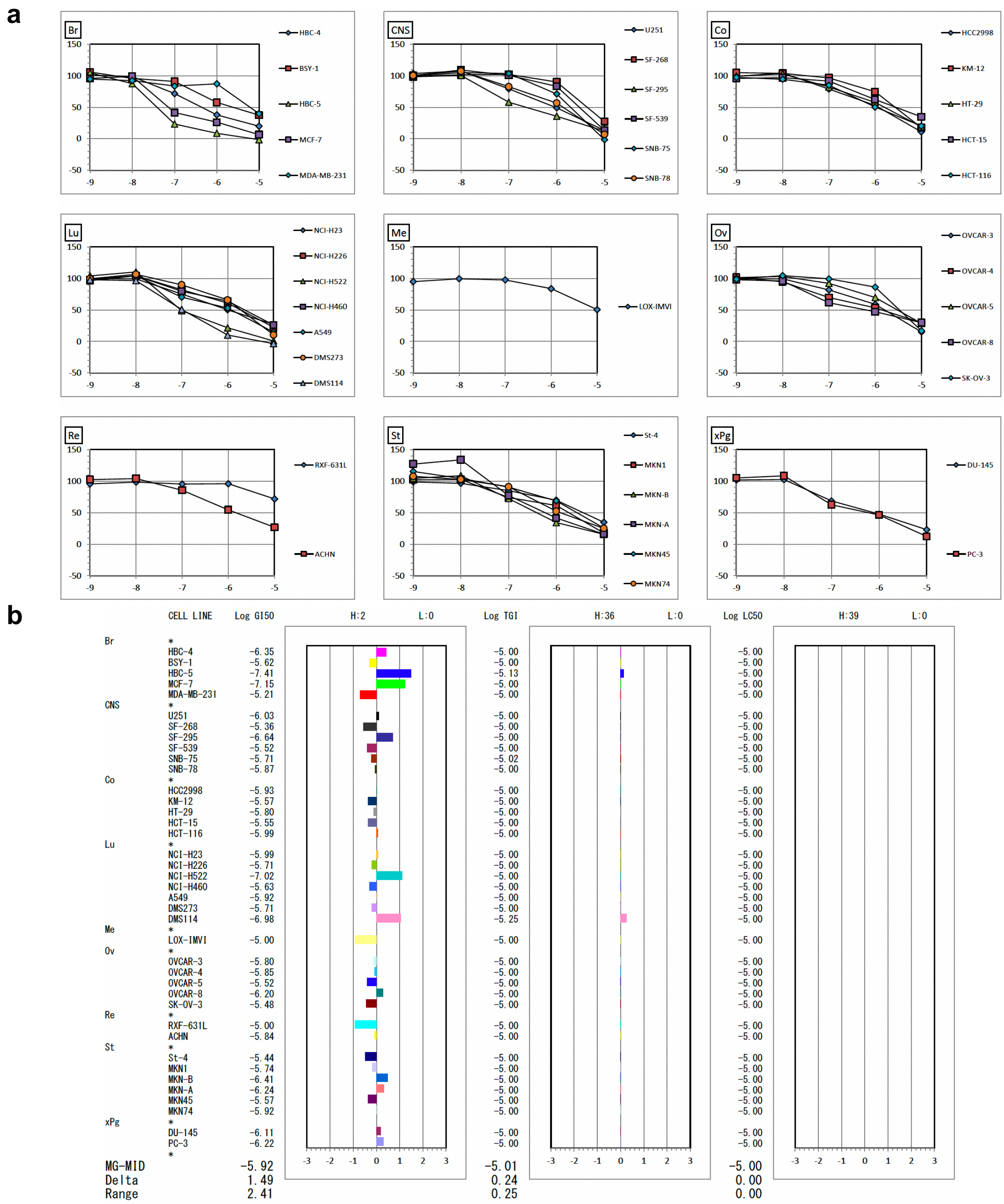


Figure S7. JFCR39 cancer cell panel assay of **1c**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1c**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1c**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 1200 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

Synthesis of 1d. The preloaded resin **8** (333 mg, 129 μmol, loading rate: 0.386 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of the standard SPPS protocol using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

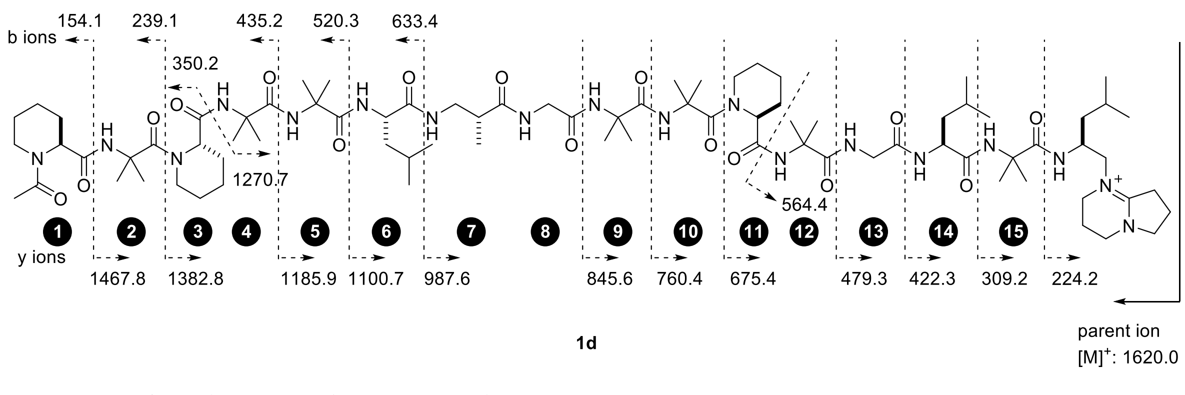
The above resin-bound peptide **18** (57.5 mg, 19.4 μmol) was subjected to 8 cycles of the standard SPPS protocol using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **11** for residue-7) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S5d**.

The resin-bound peptide **S5d** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine.

To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23d**.

To **23d** (35.1 mg, 10.8 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24d**, which was used for the next reaction without further purification.

To a solution of **24d** (10.8 μmol) in DMF (2.00 mL) in 5 mL sealed tube were added a solution of COMU (92.1 mg, 215 μmol) in DMF (1.01 mL), **7** (95.6 mg, 323 μmol), and *i*-Pr_2NEt (112 μL, 645 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give two separate peaks, peak 1 (t_R1 =25.9–28.5 min) and peak 2 (t_R2 =30.1–32.1 min), then the peaks were combined to give **1d** (773 μg, 4.1% over 32 steps): colorless foam. $[\alpha]_D^{32}$ +151 (*c* 0.023, MeOH). IR (film) 3741, 3311, 2362, 1680, 1537, 1443, 1205, 1139, 1019 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0638.



1d

Figure S8. MS/MS fragmentation pattern of **1d**.

a

b

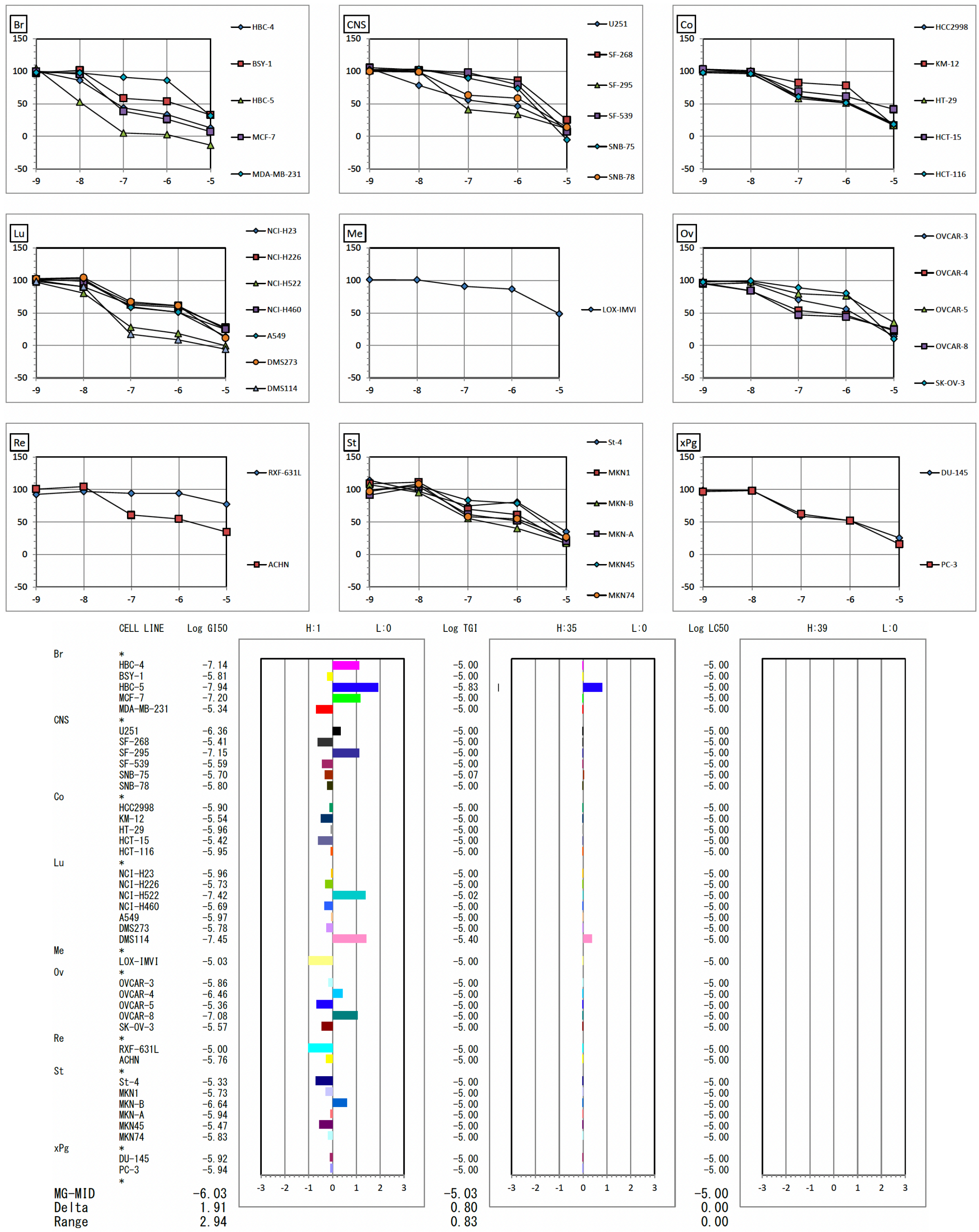


Figure S9. JFCR39 cancer cell panel assay of **1d**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1d**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1d**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 933 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

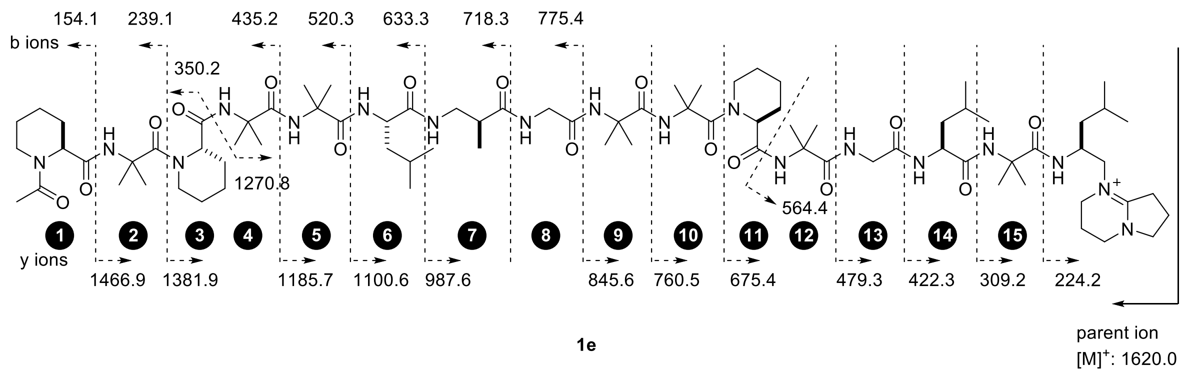
Synthesis of 1e. The preloaded resin **8** (333 mg, 129 μmol, loading rate: 0.386 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

The above resin-bound peptide **18** (62.0 mg, 20.9 μmol) was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **10** for residue-7) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S5e**.

The resin-bound peptide **S5e** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine. To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23e**.

To **23e** (30.0 mg, 9.23 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24e**, which was used for the next reaction without further purification.

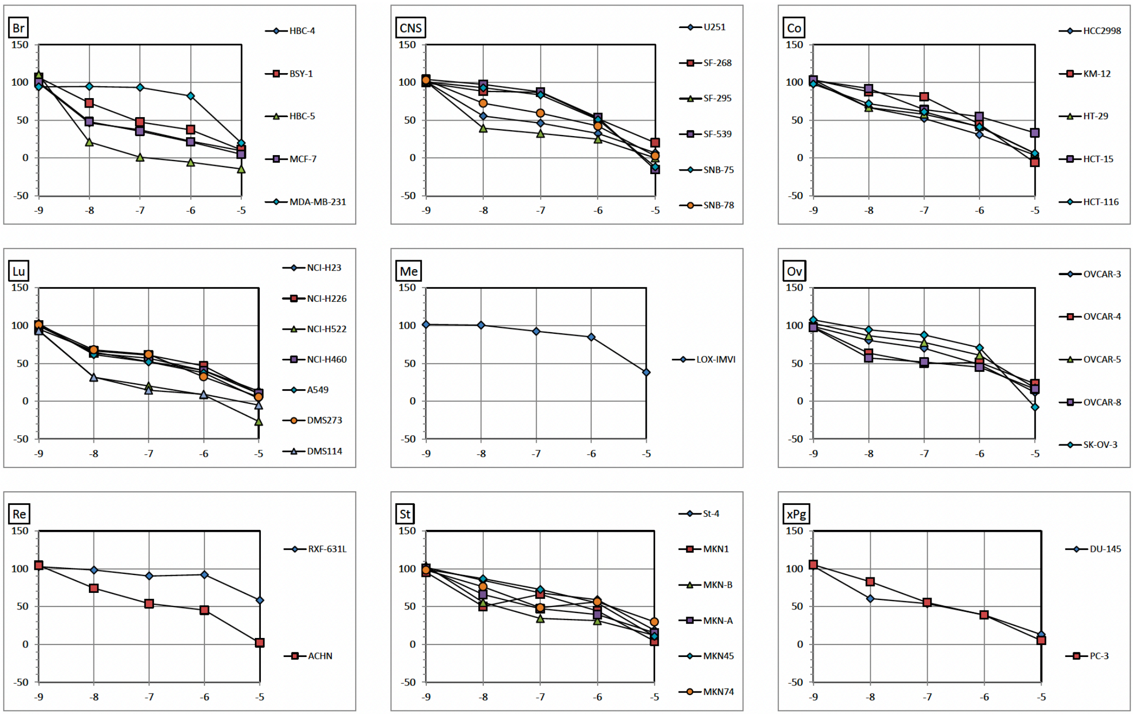
To a solution of **24e** (9.23 μmol) in DMF (2.00 mL) in 5 mL sealed tube were added a solution of COMU (79.0 mg, 185 μmol) in DMF (584 μL), **7** (82.0 mg, 277 μmol), and *i*-Pr_2NEt (96.4 μL, 554 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1e** (t_R = 44.5–47.9 min, 2.28 mg, 14% over 32 steps): colorless foam. $[\alpha]_D^{28}$ +23.7 (*c* 0.16, MeOH). IR (film) 3299, 2949, 2360, 1678, 1536, 1442, 1206, 1139, 1027, 959 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0613.



1e

Figure S10. MS/MS fragmentation pattern of **1e**.

a



b

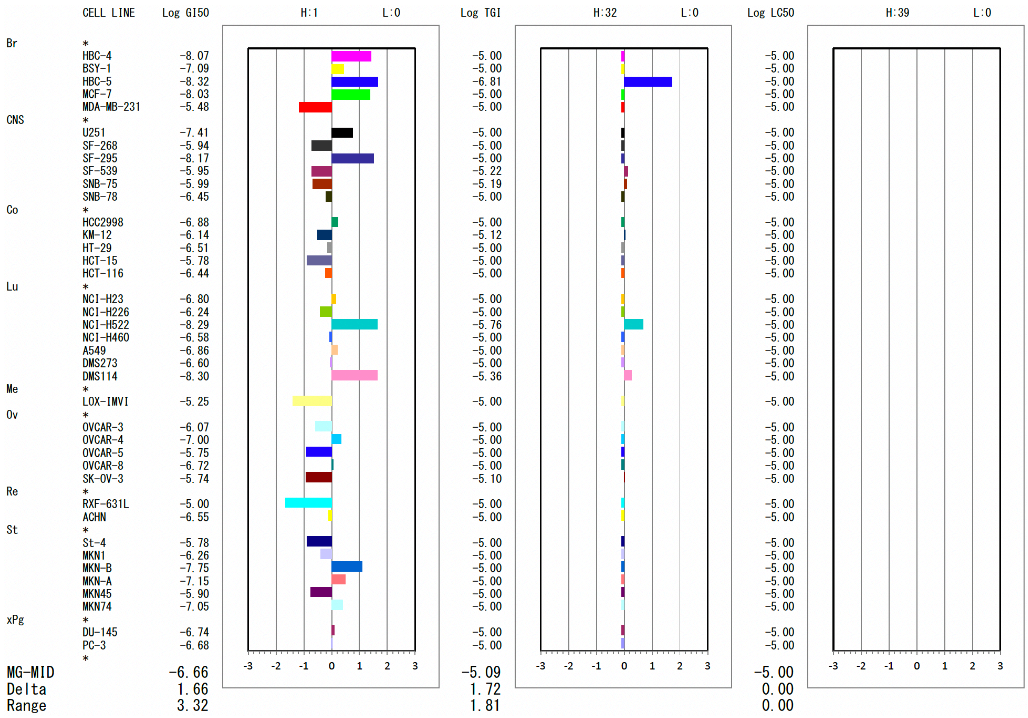


Figure S11. JFCR39 cancer cell panel assay of **1e**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1e**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1e**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 219 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

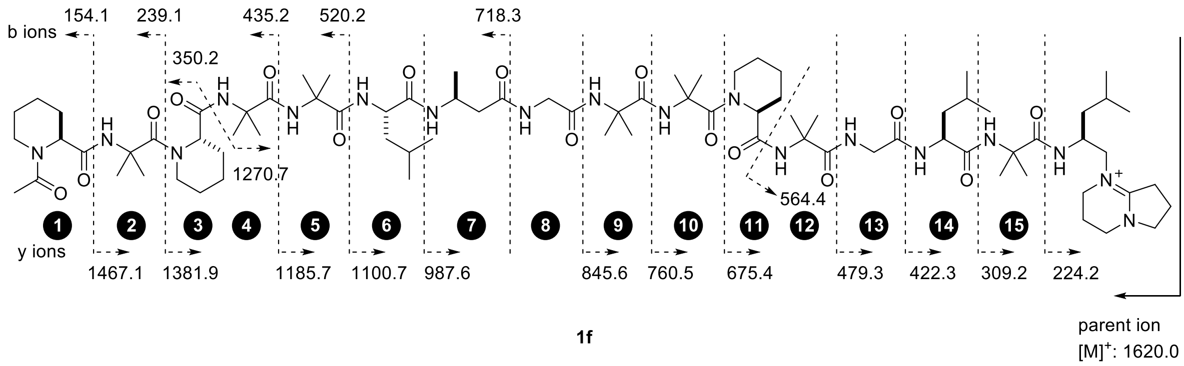
Synthesis of 1f. The preloaded resin **8** (471 mg, 158 μmol, loading rate: 0.336 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

Then the above **18** was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **12** for residue-7) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S5f**.

The resin-bound peptide **S5f** in the 20 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (4.00 mL, 40 sec × 5) and CH_2Cl_2 (4.00 mL, 40 sec × 5) to give the resin-bound amine. To the above amine in the 20 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 2.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (4.00 mL, 40 sec × 5), CH_2Cl_2 (4.00 mL, 40 sec × 5), MeOH (4.00 mL, 40 sec × 5), and Et_2O (4.00 mL, 40 sec × 5), and dried under vacuum to give **23f**.

To **23f** (314 mg, 82.2 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 2.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24f**, which was used for the next reaction without further purification.

To a solution of **24f** (82.2 μmol) in DMF (14.0 mL) was added a solution of COMU (704 mg, 1.64 mmol) in DMF (9.00 mL), **7** (731 mg, 2.47 mmol), and *i*-Pr_2NEt (859 μL, 4.93 mmol). The reaction was conducted separately in 7 batches (5 mL sealed tubes). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1f** (t_R = 45.1–50.0 min, 23.3 mg, 16% over 32 steps): colorless foam. ^1H NMR (500 MHz, DMSO-d_6): see Table S4. $^{13}C\{^1H\}$ NMR (125 MHz, DMSO-d_6): see Table S5. $[\alpha]_D^{23}$ +26.5 (*c* 0.11, MeOH). IR (film) 3853, 3747, 3673, 3649, 3614, 3294, 2941, 1435, 1055, 1010 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0638.



1f

Figure S12. MS/MS fragmentation pattern of **1f**.

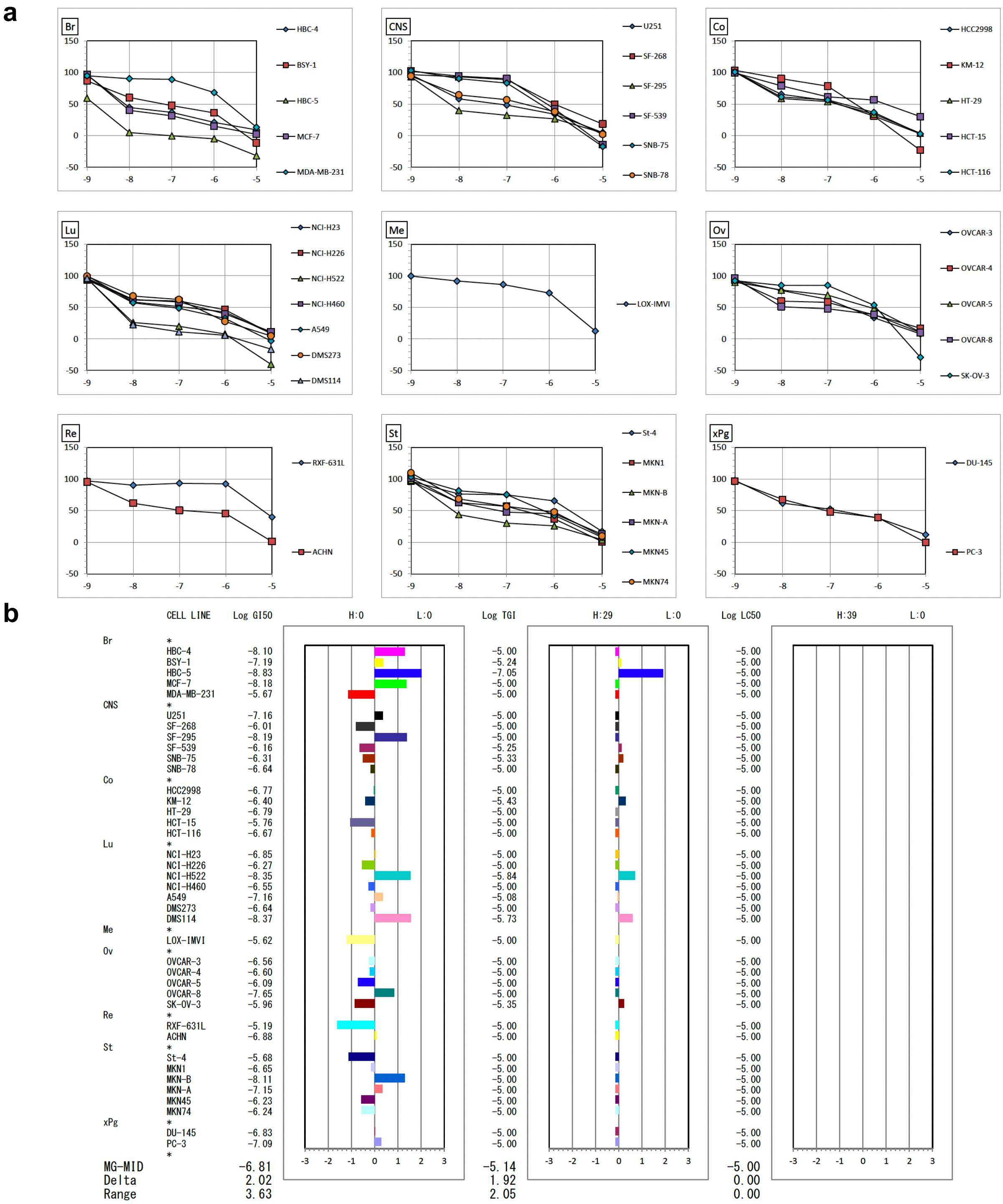


Figure S13. JFCR39 cancer cell panel assay of **1f**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1f**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI_{50}, logTGI (total growth inhibition), and logLC_{50} values of **1f**. MG-MID: mean of obtained logGI_{50} (mean GI_{50} = 155 nM), Delta: difference between logGI_{50} of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI_{50} values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

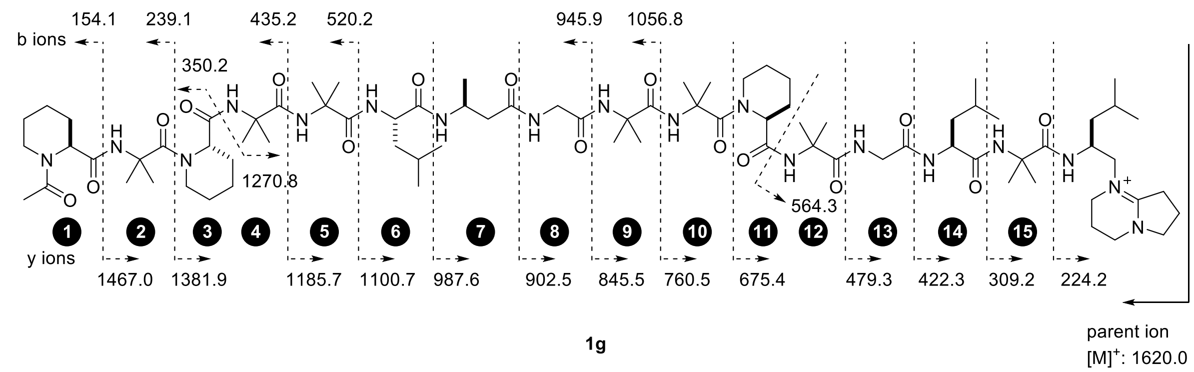
Synthesis of 1g. The preloaded resin **8** (333 mg, 129 μmol, loading rate: 0.386 mmol/g) was prepared according to the protocol described in page S5. The preloaded resin in 20 mL LibraTube was subjected to 6 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residues-12, -10, and -9, **5** for residue-11) to give the resin-bound heptapeptide **18**.

The above resin-bound peptide **18** (85.7 mg, 28.9 μmol) was subjected to 8 cycles of standard operation A or B using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-5, -4, and -2, **5** for residues-3 and -1, **13** for residue-7) to give the 15-mer N_{α}-Fmoc-protected resin-bound peptide **S5g**.

The resin-bound peptide **S5g** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2), and washed with NMP (2.00 mL, 40 sec × 5) and CH_2Cl_2 (2.00 mL, 40 sec × 5) to give the resin-bound amine. To the above amine in the 5 mL LibraTube was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **23g**.

To **23g** (34.7 mg, 11.7 μmol) was added $(CF_3)_2CHOH/CH_2Cl_2$ (1/3, 1.00 mL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude **24g**, which was used for the next reaction without further purification.

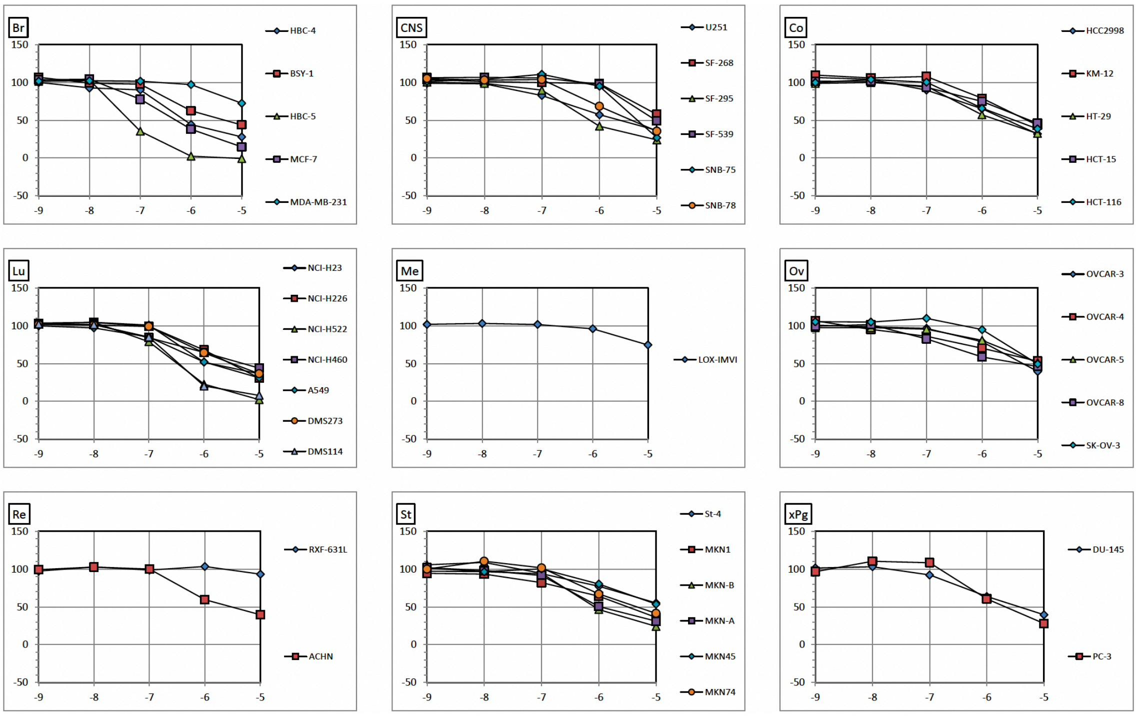
To a solution of **24g** (11.7 μmol) in DMF (2.50 mL) in 5 mL sealed tube were added a solution of COMU (100 mg, 233 μmol) in DMF (767 μL), **7** (104 mg, 350 μmol), and *i*-Pr_2NEt (122 μL, 700 μmol). After being stirred at 30 °C for 72 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 2.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1g** (t_R = 42.0–46.7 min, 2.94 mg, 15% over 32 steps): colorless foam. $[\alpha]_D^{28}$ −10.8 (*c* 0.078, MeOH). IR (film) 3845, 3741, 3674, 3649, 3622, 2376, 2314, 1537, 1452, 1207, 1814, 1139 cm^{-1}. HRMS (ESI-TOF) m/z: Calcd for $C_{81}H_{139}N_{18}O_{16}^+$ $[M]^+$ 1620.0611; Found 1620.0588.



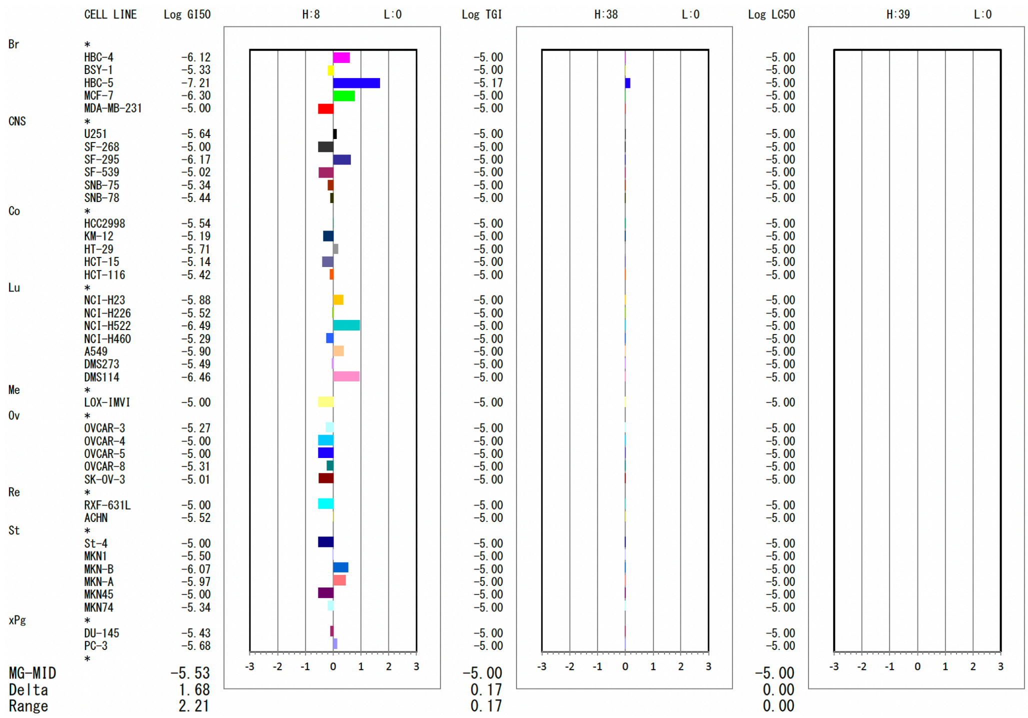
1g

Figure S14. MS/MS fragmentation pattern of **1g**.

a



b



	CELL LINE	Log GI50	Log TGI	Log LC50
Br	*			
	HBC-4	-6.12	-5.00	-5.00
	BSY-1	-5.33	-5.00	-5.00
	HBC-5	-7.21	-5.17	-5.00
	MCF-7	-6.30	-5.00	-5.00
	MDA-MB-231	-5.00	-5.00	-5.00
CNS	*			
	U251	-5.64	-5.00	-5.00
	SF-268	-5.00	-5.00	-5.00
	SF-295	-6.17	-5.00	-5.00
	SF-539	-5.02	-5.00	-5.00
	SNB-75	-5.34	-5.00	-5.00
	SNB-78	-5.44	-5.00	-5.00
Co	*			
	HCC2998	-5.54	-5.00	-5.00
	KM-12	-5.19	-5.00	-5.00
	HT-29	-5.71	-5.00	-5.00
	HCT-15	-5.14	-5.00	-5.00
	HCT-116	-5.42	-5.00	-5.00
Lu	*			
	NCI-H23	-5.88	-5.00	-5.00
	NCI-H226	-5.52	-5.00	-5.00
	NCI-H522	-6.49	-5.00	-5.00
	NCI-H460	-5.29	-5.00	-5.00
	A549	-5.90	-5.00	-5.00
	DMS273	-5.49	-5.00	-5.00
	DMS114	-6.46	-5.00	-5.00
Me	*			
	LOX-IMVI	-5.00	-5.00	-5.00
Ov	*			
	OVCAR-3	-5.27	-5.00	-5.00
	OVCAR-4	-5.00	-5.00	-5.00
	OVCAR-5	-5.00	-5.00	-5.00
	OVCAR-8	-5.31	-5.00	-5.00
	SK-OV-3	-5.01	-5.00	-5.00
Re	*			
	RXF-631L	-5.00	-5.00	-5.00
	ACHN	-5.52	-5.00	-5.00
St	*			
	St-4	-5.00	-5.00	-5.00
	MKN1	-5.50	-5.00	-5.00
	MKN-B	-6.07	-5.00	-5.00
	MKN-A	-5.97	-5.00	-5.00
	MKN45	-5.00	-5.00	-5.00
	MKN74	-5.34	-5.00	-5.00
xPg	*			
	DU-145	-5.43	-5.00	-5.00
	PC-3	-5.68	-5.00	-5.00
	*			
MG-MID		-5.53	-5.00	-5.00
Delta		1.68	0.17	0.00
Range		2.21	0.17	0.00

Figure S15. JFCR39 cancer cell panel assay of **1g**. (a) Concentration-response curves obtained by sulforhodamine B assays. The cells were incubated for 2 d in the presence of **1g**, and the cell growth (%) was evaluated. Each plot is displayed as mean of two replicates. (b) Obtained logGI50, logTGI (total growth inhibition), and logLC50 values of **1g**. MG-MID: mean of obtained logGI50 (mean GI_{50} = 2950 nM), Delta: difference between logGI50 of the cell line exhibiting the highest sensitivity and MG-MID, Range: difference between logGI50 values of the cell lines exhibiting the highest sensitivity and lowest sensitivity, TGI: concentrations that cause cell growth (%) = 0%.

Table S1. Pearson correlation coefficients (r values) of GI_{50} values of **1b–1g** in COMPARE analysis[S3] with **1a**

compounds	R
1b	0.898
1c	0.877
1d	0.870
1e	0.893
1f	0.862
1g	0.850

Attempted synthesis of 24a using Wang-ChemMatrix resin.

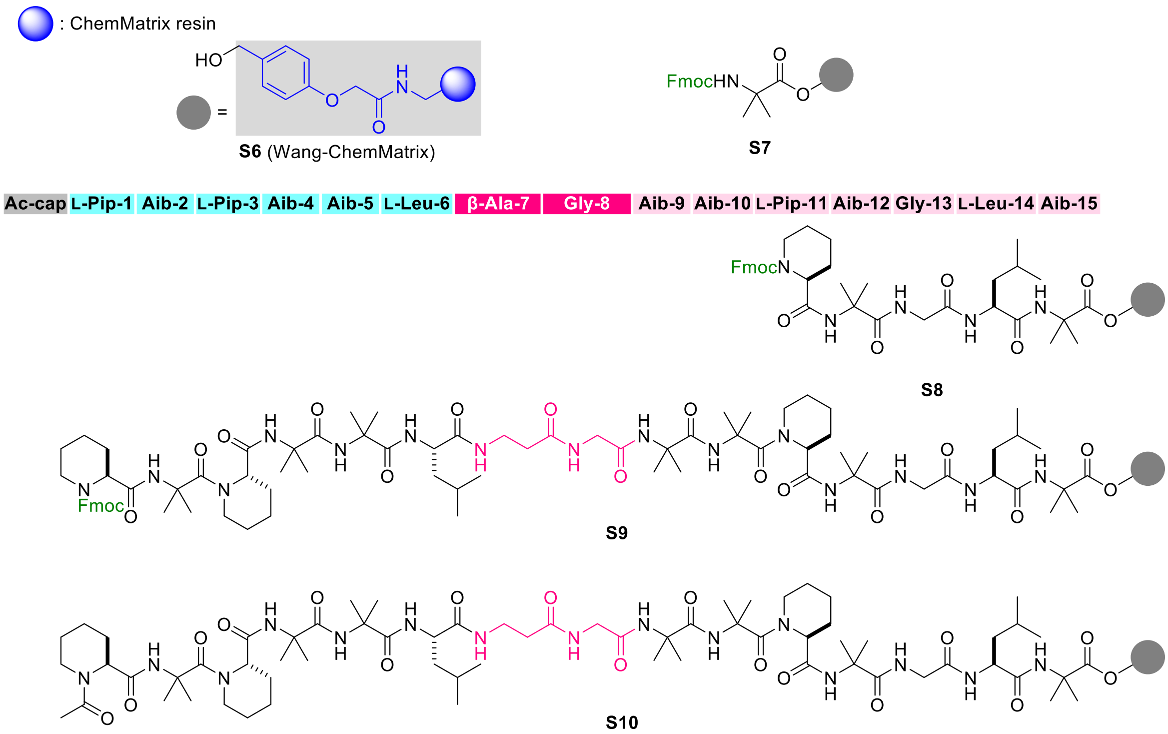


Figure S16. Structures of **S6**–**S10**.

Preloaded resin S7. To Wang-ChemMatrix resin (**S6**, 171 mg, 80.3 μmol) in 20 mL LibraTube (Hipep Laboratories) was washed with CH_2Cl_2 (4.00 mL × 5) and CH_2Cl_2/NMP (4.00 mL × 5). Then a solution of **4** (104 mg, 321 μmol), *N*,*N*'-diisopropylcarbodiimide (37.7 μL, 241 μmol) and 4-dimethylaminopyridine (2.94 mg, 24.1 μmol) in CH_2Cl_2 (1.33 mL) was added to **S6**. After being stirred at room temperature for 14 h, the reaction mixture was filtered. The resultant resin was washed with CH_2Cl_2/NMP (4.00 mL × 5), CH_2Cl_2 (4.00 mL × 5). To the above resin was added Ac_2O (**6**)/CH_2Cl_2 (1/3, 2.00 mL) at room temperature for capping the remaining hydroxy groups. After being stirred at room temperature for 15 min, the reaction mixture was filtered, washed with CH_2Cl_2 (4.00 mL, 40 sec × 5), NMP (4.00 mL, 40 sec × 5), MeOH (4.00 mL, 40 sec × 5), and Et_2O (4.00 mL, 40 sec × 5), and dried under vacuum to give preloaded resin **S7** (187 mg). The loading rate of **S7** was determined to be 0.297 mmol/g by the protocol described in page S5.

Procedures for solid-phase peptide synthesis (SPPS). The resin-bound peptides **S10** were prepared on a peptide synthesizer MWS-1000 (EYELA).

Standard operation C for conjugating **2** for residues-14 and -6, **3** for residue-13, **4** for residues-12 and -5, **5** for residues-11, -3, and -1, **9** for residue-8, and **3** for residue-7 was shown as follows:

Step 1: The solid supported N_α-Fmoc peptide was deprotected with piperidine/NMP (1/4, 40 °C, 5 min × 2).

Step 2: The resin in a reaction vessel (5 mL LibraTube) was washed with NMP (2.00 mL, 40 sec × 5).

Step 3: An N_α-Fmoc-protected amino acid (4.0 eq) in a vial was activated by a solution of *O*-(7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 4.0 eq, 0.45 M)/1-hydroxy-7-azabenzotriazole (HOAt, 4.0 eq, 0.45 M) in NMP. To the solution of activated amino acid was added a solution of *i*-Pr_2NEt (8.0 eq, 2.0 M) in NMP. The resultant mixture was transferred to the reaction vessel.

Step 4: The activated N_α-Fmoc-protected amino acid was coupled with the peptide on the resin (60 °C, 20 min), and the reaction vessel containing the resin was washed with NMP (2.00 mL, 40 sec × 5).

Steps 1−4 were repeated and amino acids were condensed on the solid support. For residues-12 and -5, Steps 3 and 4 were repeated (×2). Steps 2 and 4 were carried out under a stream of N_2. Steps 1 and 3 were carried out under atmosphere of N_2.

Standard operation D for conjugating **4** for residues-10, -9, -4, and -2 was shown as follows:

Step 1: The solid supported N_α-Fmoc peptide was deprotected with piperidine/NMP (1/4, 40 °C, 5 min × 2).

Step 2: The resin in a reaction vessel (5 mL LibraTube) was washed with NMP (2.00 mL, 40 sec × 5).

Step 3: N_α-Fmoc-protected Aib **4** (4.0 eq) in a vial was activated by a solution of 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU, 4.0 eq, 0.4 M) in NMP. To the solution of activated amino acid was added a solution of *i*-Pr_2NEt (8.0 eq). The resultant mixture was transferred to the reaction vessel.

Step 4: The activated N_α-Fmoc-protected amino acid was coupled with the peptide on the resin (room temperature, 2 h), and the reaction vessel containing the resin was washed with NMP (2.00 mL, 40 sec × 5).

Steps 1−4 were repeated and amino acids were condensed on the solid support. Steps 3 and 4 were repeated (×3).

Attempted synthesis of 24a from S7. The preloaded resin **S7** (91.0 mg, 27.0 μmol, loading rate: 0.297 mmol/g) was prepared according to the protocol described in page S23. The preloaded resin in 5 mL LibraTube was subjected to 4 cycles of standard operation C or D using Fmoc-protected amino acids (**2** for residue-14, **3** for residue-13, **4** for residue-12, **5** for residue-11) to give the resin-bound pentapeptide **S8** (97.6 mg).

The above resin-bound peptide **S8** (45.3 mg, 12.5 μmol) was subjected to 10 cycles of standard operation C or D using Fmoc-protected amino acids (**2** for residue-6, **3** for residue-8, **4** for residues-10, -9, -5, -4, and -2, **5** for residues-3 and -1, **9** for residue-7) to give the 15-mer N_α-Fmoc-protected resin-bound peptide **S9**.

The resin-bound peptide **S9** in the 5 mL LibraTube was treated with piperidine/NMP (1/4, 40 °C, 5 min × 2) and washed with NMP (2.00 mL, 40 sec × 5) to give the resin-bound amine.

AcOH (4.0 eq) in a vial was activated by a solution of *O*-(7-azabenzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 4.0 eq, 0.45 M)/1-hydroxy-7-azabenzotriazole (HOAt, 4.0 eq, 0.45 M) in NMP. To the solution of activated AcOH was added a solution of *i*-Pr_2NEt (8.0 eq, 2.0 M) in NMP. The resultant mixture was transferred to the reaction vessel.

The activated AcOH was coupled with the peptide on the resin (60 °C, 20 min). The reaction mixture was filtered.

The condensation of AcOH was repeated. The reaction mixture was filtered, and washed with NMP (2.00 mL, 40 sec × 5), CH_2Cl_2 (2.00 mL, 40 sec × 5), MeOH (2.00 mL, 40 sec × 5), and Et_2O (2.00 mL, 40 sec × 5), and dried under vacuum to give **S10** (39.1 mg).

To **S10** (0.42 mg, 0.115 μmol) was added TFA/H_2O (95/5, 100 μL) at room temperature. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under a stream of Ar to give the crude material **A**. The crude material **A** was dissolved into MeOH and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was analyzed by LC-MS (Figure S17, column: Inertsil C8-3 4.6 × 150 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 40/60 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 220 nm, temperature: 40 °C).

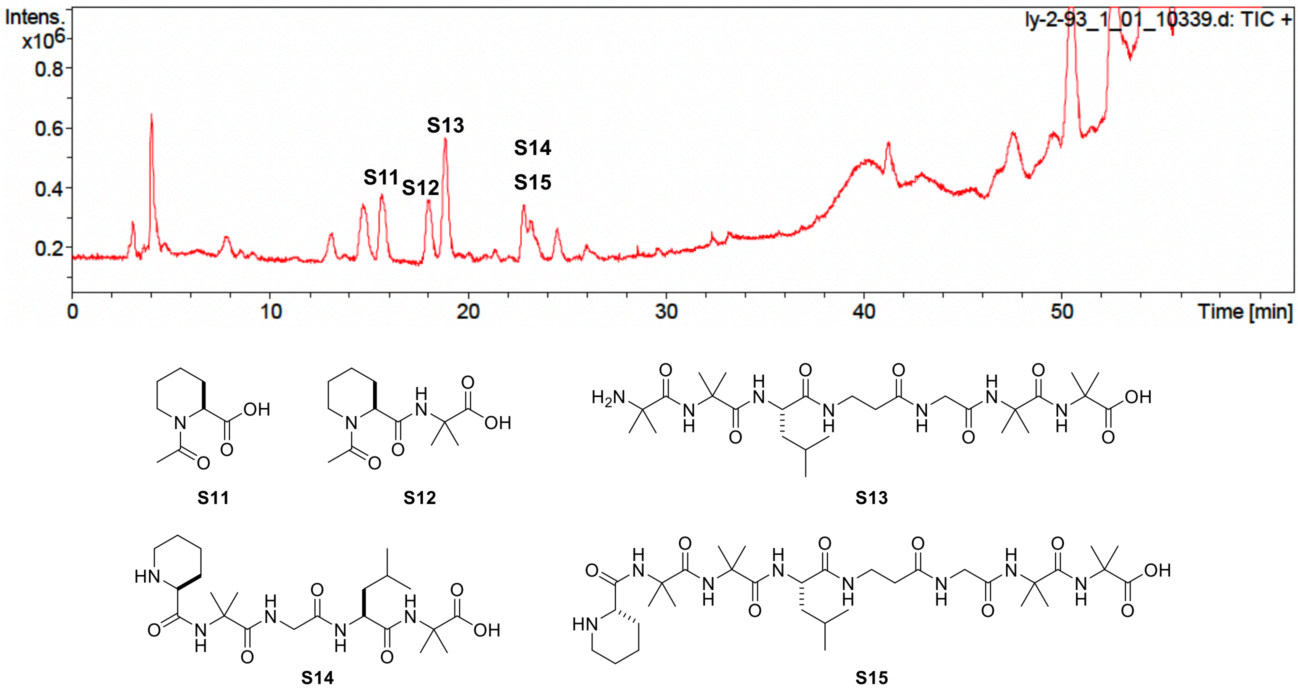


Figure S17. Total ion chromatogram of the crude material **A** described in page S25. The structures of **S11**–**S15** were tentatively assigned based on mass spectra. Column: Inertsil C8-3 4.6 × 150 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 40/60 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

Investigation of condensing agents for forming a sterically demanding amide bond.

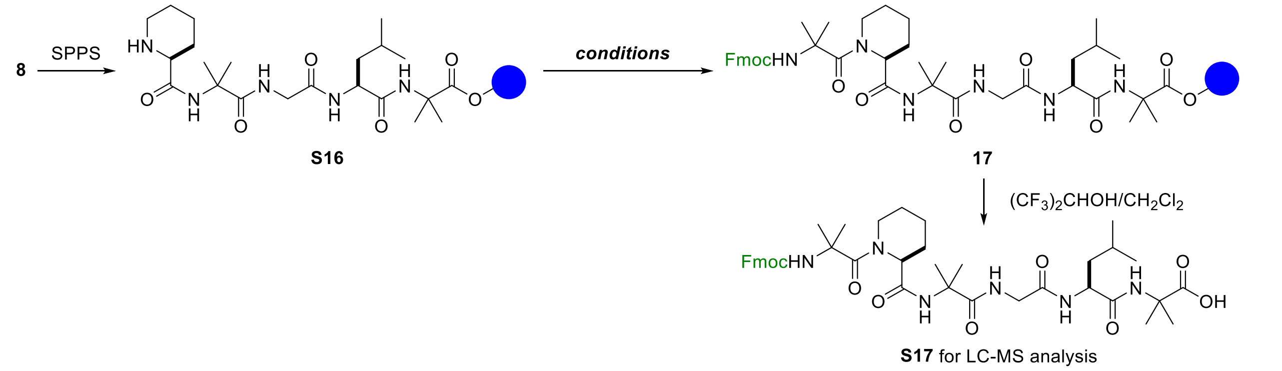


Table S2. Condensation of Fmoc-Aib-OH (**4**) with L-Pip-11 of resin-bound **S16**

entry	conditions	yield of **S17** from **8**[a]
1	**4** (4.0 eq), HATU (4.0 eq), HOAt (4.0 eq), *i*-Pr_2NEt (8.0 eq) NMP (0.078 M), 60 ºC, 20 min (2 cycles)	13% (Figure S18)
2	**4** (4.0 eq), COMU (4.0 eq), *i*-Pr_2NEt (8.0 eq) NMP (0.10 M), 40 ºC, 2 h (2 cycles)	74% (Figure S19)

[a]The yield was calculated from HPLC chromatogram (UV 300 nm). The amount of **S17** was estimated from its peak area using the peak of Fmoc-Gly-OH as the reference.

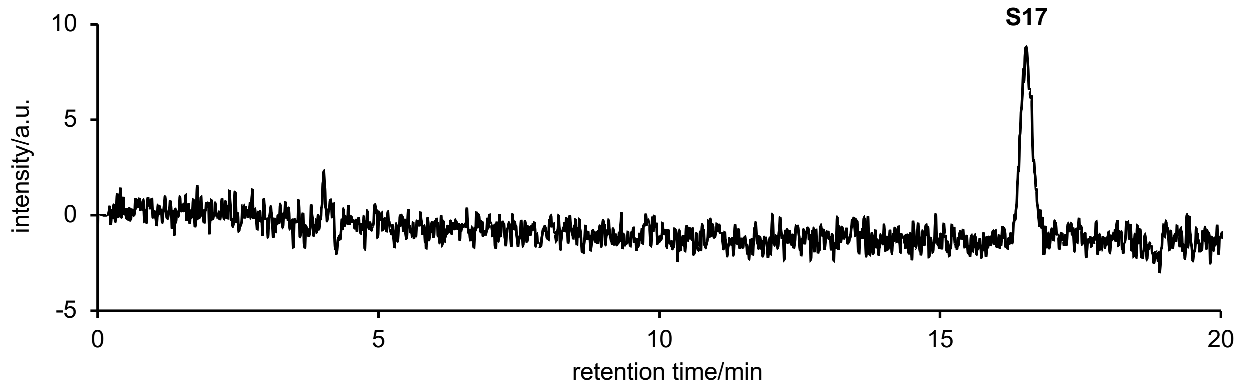


Figure S18. HPLC chart for quantification of **S17** (Table S2, entry 1). Column: Inertsil C8-3 4.6 × 150 mm, eluent A: eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 60/40 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 300 nm, temperature: 40 ºC.

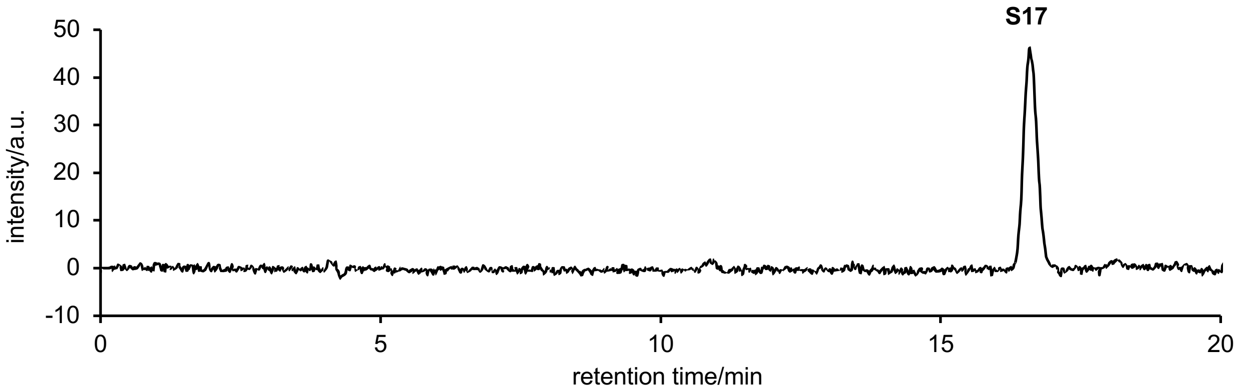


Figure S19. HPLC chart for quantification of **S17** (Table S2, entry 2). Column: Inertsil C8-3 4.6 × 150 mm, eluent A: eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 60/40 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 300 nm, temperature: 40 ºC.

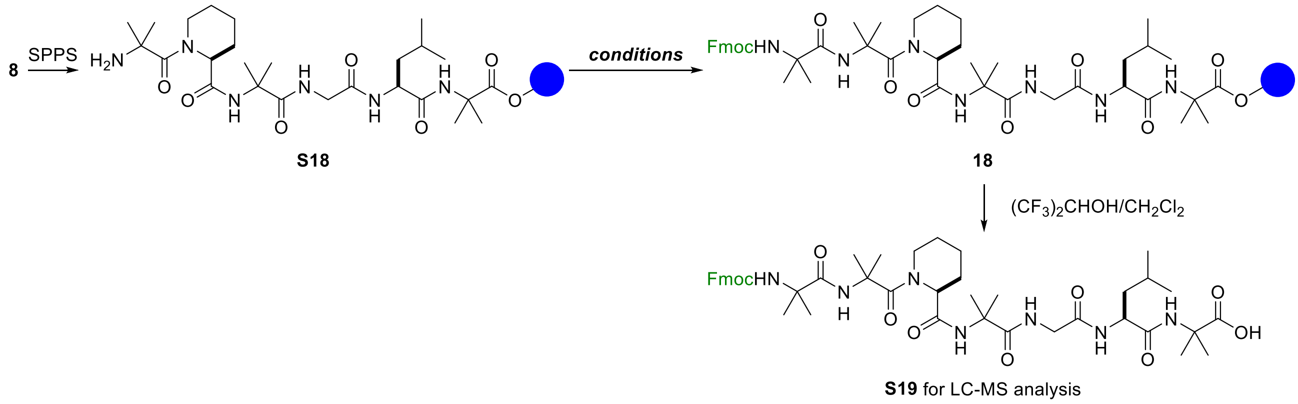


Table S3. Condensation of Fmoc-protected Aib with N-terminal Aib in resin-bound **S18**

entry	conditions	yield of **S19** from **8**[a]
1	Fmoc-Aib-OH (4.0 eq), HATU (4.0 eq), HOAt (4.0 eq), *i*-Pr_2NEt (8.0 eq) NMP (0.078 M), 60 ºC, 20 min (2 cycles)	61% (Figure S20)
2	Fmoc-Aib-OH (4.0 eq), COMU (4.0 eq), *i*-Pr_2NEt (8.0 eq) NMP (0.10 M), 40 ºC, 2 h (2 cycles)	>99% (Figure S21)

[a]The yield was calculated from HPLC chromatogram (UV 300 nm). The quantity of **S19** was determined from its peak area using the peak of Fmoc-Gly-OH as the reference.

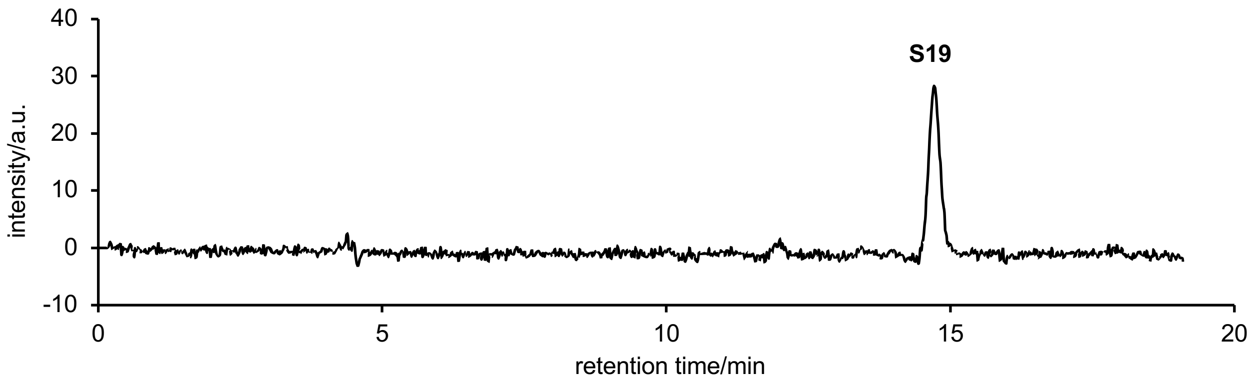


Figure S20. HPLC chart for quantification of **S19** (Table S3, entry 1). Column: Inertsil C8-3 4.6 × 150 mm, eluent A: eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 60/40 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 300 nm, temperature: 40 ºC.

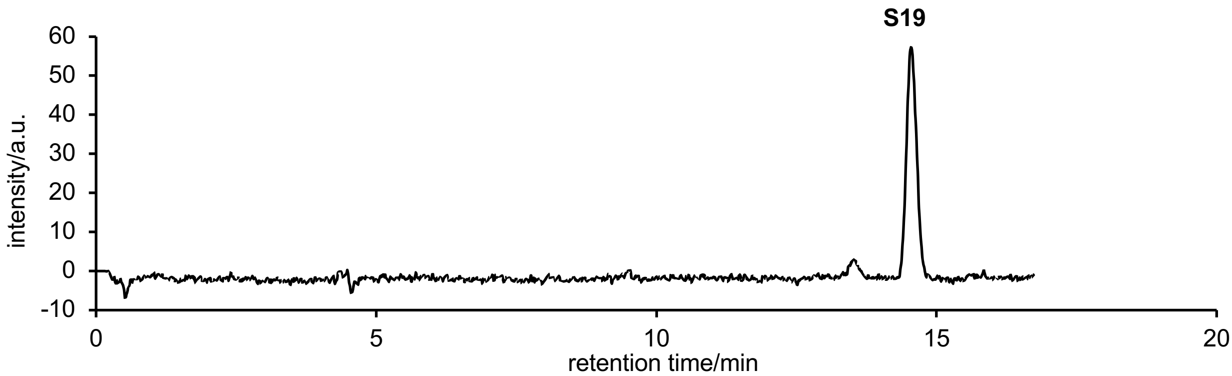
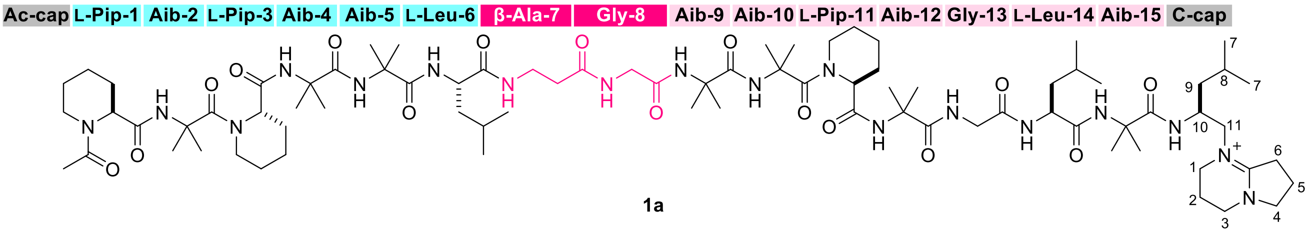


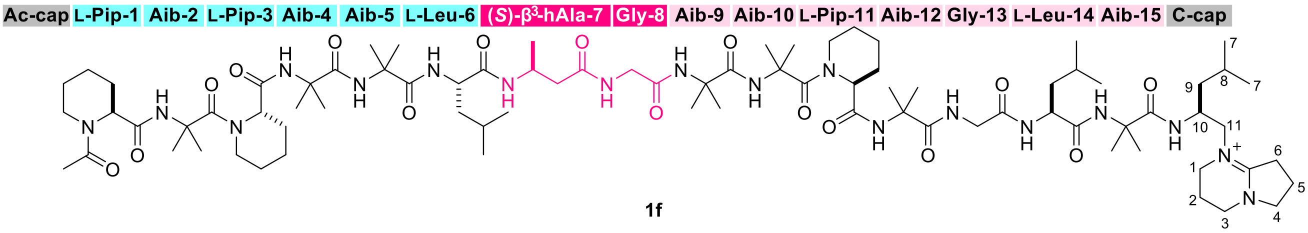
Figure S21. HPLC chart for quantification of **S19** (Table S3, entry 2). Column: Inertsil C8-3 4.6 × 150 mm, eluent A: eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 60/40 to 100/0 over 40 min, flow rate: 1.50 mL/min, detection: UV 300 nm, temperature: 40 ºC.

Attempted introduction of C-cap. Compound **24a** was prepared according to the protocol described in page S8. To a solution of **7** (1.06 mg, 3.57 μmol) in DMF (10.0 μL) was added Et_3N (0.498 μL, 3.57 μmol). After removing $Et_3N \cdot HCl$ by centrifugation at 112 × *g* for 5 min at room temperature, the corresponding free amine was obtained from the supernatant. To a solution of **24a** (0.500 mg, 0.357 μmol) in DMF (70.0 μL) in 1 mL sealed tube were added a solution of COMU (1.53 mg, 3.57 μmol) in DMF (10.0 μL), a solution of the above free amine (3.57 μmol) in DMF (10.0 μL), and *i*-Pr_2NEt (1.24 μL, 7.14 μmol). After being stirred at room temperature for 4 h, the reaction mixture was diluted with *i*-PrOH/H_2O (8/2, 1.00 mL), and filtered through a PTFE filter with 0.20 μm of pore size in the diameter. The filtrate was purified by reversed-phase HPLC (column: Inertsil C8-3 4.6 × 150 mm, eluent A: *i*-PrOH, eluent B: H_2O, linear gradient A/B = 90/10 to 100/0 over 60 min, flow rate: 0.500 mL/min, detection: UV 220 nm, temperature: 40 °C) to give **1a** (23.5 μg, 3.9% over 32 steps). The quantity of **1a** was determined from the ^{1}H NMR spectrum using Fmoc-L-Val-OH as an external standard.

Table S4. ^{1}H NMR (500 MHz) chemical shifts of **1a** and **1f**^{a}



1a



1f

residue	position	δ_H (reported **1a**)[S4]	δ_H (synthetic **1a**)	δ_H (**1f**)
Ac-cap	α	no data	2.05	2.05
L-Pip-1	α	5.04	5.03	5.02
	β	1.49/2.11	1.49/1.53	1.50/2.13
	γ	1.34	1.33	1.34
	δ	3.00	2.15	2.20
	ε	3.37	4.49	4.53
Aib-2	β	1.41	1.39	1.40
	NH	8.25	8.25	8.32
L-Pip-3	α	4.98	4.97	4.98
	β	1.46/2.05	1.44/2.04	1.44/2.04
	γ	1.36	1.53	1.50
	δ	2.98	3.00	2.96
	ε	4.10	4.08	4.11
Aib-4	β	no data	1.39	1.39
	NH	7.67	7.67	7.70
Aib-5	β	1.30/1.40	1.31/1.40	1.29
	NH	7.96	7.95	8.03
L-Leu-6	α	4.01	4.00	3.97
	β	1.63	1.60	1.64
	γ	no data	1.47	1.33
	δ	no data	0.78/0.87	0.80
	NH	7.54	7.54	7.54
β-Ala-7 for **1a**/ (*S*)-β^{3}-hAla-7 for **1f**	α	4.02	3.25	4.11
	β	3.26	2.32/2.37	2.23/2.41/1.05
	NH	7.53	7.53	7.32
Gly-8	α	3.59	3.58	3.36/3.64
	NH	8.33	8.34	8.14
Aib-9	β	1.35/1.41	1.35/1.42	1.35/1.40
	NH	8.13	8.17	8.04
Aib-10	β	no data	1.45	1.39
	NH	7.67	7.71	7.78
L-Pip-11	α	5.35	5.34	5.32
	β	1.30/2.19	1.30/2.19	1.31/2.18
	γ	1.31	1.51	1.50
	δ	2.73	2.71	2.73
	ε	4.22	4.20	4.22
Aib-12	β	1.38/1.44	1.35/1.40	1.42
	NH	7.65	7.66	7.64

(continued)

residue	position	δ_H (reported **1a**)	δ_H (synthetic **1a**)	δ_H (**1f**)
Gly-13	α	3.61/3.69	3.60/3.70	3.65
	NH	8.45	8.44	8.44
L-Leu-14	α	3.93	3.93	3.93
	β	1.47/1.66	1.47/1.66	1.45/1.63
	γ	no data	1.15	1.14
	δ	no data	0.83/0.89	0.82/0.87
	NH	7.85	7.86	7.86
Aib-15	β	1.36	1.33	1.35
	NH	7.36	7.36	7.38
C-cap	1	no data	2.90 or 3.67	2.36 or 3.67
	2	no data	or 2.10	or 2.04 or 2.10
	3	no data	or 3.17 for positions 1, 2, 3	or 3.23 for positions 1, 2, 3
	4	no data	2.02 or 2.92	1.95 or 2.95
	5	no data	or 3.70 or 3.18	or 3.30 or 3.17
	6	no data	or 3.49 for positions 4, 5, 6	or 3.35 for positions 4, 5, 6
	7	no data	0.81/0.87	0.71/0.85
	8	1.16	1.15	1.15
	9	1.40/1.49	1.47/1.50	1.47/1.51
	10	4.21	4.20	4.18
	11	3.31/3.54	3.35	3.33
	NH	6.96	6.96	6.93

[a]The spectra of **1a** (2.98 mM) and **1f** (67.2 mM) were obtained in DMSO-d_6 at 27 °C.

Table S5. $^{13}C\{^{1}H\}$ NMR (125 MHz) chemical shifts of **1a** and **1f**[a]

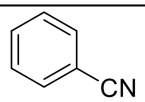
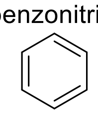
δ_C (synthetic **1a**)	δ_C (**1f**)	δ_C (synthetic **1a**)	δ_C (**1f**)
17.6	17.6	55.9	54.0
18.3	18.3	56.0 (2C)	54.1
19.7	19.8	56.1	55.9
20.1	20.2	56.2	56.1 (2C)
20.5 (2C)	20.5 (2C)	56.2	56.2
21.0 (2C)	21.1 (2C)	56.2	56.3 (2C)
21.1 (2C)	21.2 (2C)	56.3	56.3
21.6	21.7	164.5	56.4
22.8 (2C)	22.8	168.7	164.6
23.0	22.9 (2C)	170.0	168.7
23.1 (2C)	23.1 (2C)	170.9	170.1
23.3 (2C)	23.3 (2C)	171.2	171.0
23.5	23.6	171.2	171.2
23.7	23.7	171.4	171.3
24.1	24.1	171.7	171.4
24.1	24.2	171.8	171.5
24.4 (2C)	24.4 (2C)	171.9	171.8
24.5	24.6	172.2	171.9
24.7 (2C)	24.7 (2C)	172.4	172.0
24.9	24.9	174.0	172.5
25.1	25.0	174.3	174.1
25.2 (2C)	25.2	174.8	174.6
25.4	25.4	175.0	174.9
25.8	25.5	175.4	175.1
26.1	25.8		175.5
26.3	26.2		
26.8 (2C)	26.3		
30.2	26.9 (2C)		
35.1	26.9		
35.5	27.1		
39.4	30.2		
40.2	39.4		
41.9	40.3		
42.7	41.9		
43.1	42.4		
43.5	42.7		
43.5	43.4		
43.8	43.6		
43.9	43.8		
48.5	43.9		
51.5	48.6		
51.8	51.6		
52.7	52.2		
53.0	52.9		
53.9	53.1		

[a]The spectra of **1a** (36.9 mM) and **1f** (67.2 mM) were obtained in DMSO-d_6 at 27 °C.

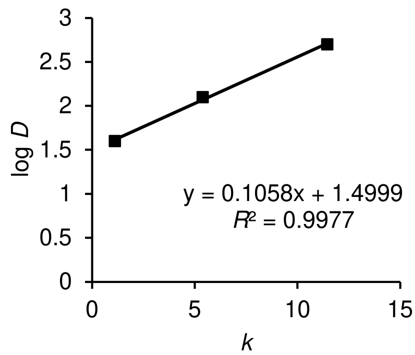
Computational simulations. The conformational parameters were calculated during replica exchange with solute tempering (REST) simulations by Desmond (Schrödinger) using the OPLS3e force field. The initial structures of **1a**–**1g** were generated based on the reported crystal structure of **1a** with F_1 domain of F_oF_1-ATP synthase (PDB ID: 1EFR). The production MD was performed in the NPT ensemble at 300 K and 1.01325 bar. The simulations were performed for 40 ns using 6 replicas with an effective temperature of the solute from 300 to 433 K (for **1a**) or 300 to 430 K (for **1b**–**1g**), determined by setting the acceptance ratio for exchange to 0.3. The trajectory structures were saved every 20 ps (total frames: 2000).

CD measurement. The purified peptides **1a**–**1g** (24.4 nmol) were dissolved in CF_3CH_2OH (400 μL). CD spectra were recorded in a 0.2 cm pathlength cuvette at 27 °C using a J-820 spectropolarimeter equipped with Peltier thermostatted cell holder (JASCO). The data were acquired for 200–260 nm at every 0.2 nm with a standard sensitivity mode (100 mdeg) applying 2 nm band width. Each measurement was repeated four times at a scanning speed of 50 nm/min with a response time of 1 sec at 27 °C. In all cases, the peptide-free solvent spectra were subtracted from the peptide solution spectra. Intensity of each CD spectrum was expressed in units of molar ellipticity (deg cm^2 $dmol^{-1}$) and plotted against the wavelength (nm).

Log *D* evaluation. Log *D* values of **1a**–**1g** were evaluated by UHPLC (column: Accucore C18 2.1 mm × 150 mm; eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: UV 220, 254 nm, temperature: 40 ºC). The column dead time was determined by injection of $NaNO_3$ as a non-retained marker, and its retention time (t_0) was 1.51 min. The standard curve was obtained by plotting the capacity factor [$k = (t_R - t_0)/t_0$] of the three standard compounds [benzonitrile (t_R = 3.18 min), benzene (t_R = 9.64 min), and toluene (t_R = 18.81 min)] versus the known log *D* values. The log *D* values of **1a**–**1g** were calculated from the retention times.

standard sample	t_R/min	k^a	log *D*
$NaNO_3$	1.51 (= t_0)	-	-
 benzonitrile	3.19	1.11	1.6
 benzene	9.64	5.39	2.1
 toluene	18.81	11.46	2.7

$^a k = (t_R - t_0)/t_0$



sample	t_R/min	k^a	log *D*
1a	9.04	4.99	2.03
1b	14.71	8.74	2.43
1c	9.25	5.13	2.04
1d	10.14	5.72	2.11
1e	13.57	7.98	2.35
1f	16.42	9.87	2.54
1g	7.62	4.05	1.93

Figure S22. Determination of log *D* values of **1a**–**1g**. Data of standard samples, a standard curve for calculating log *D* values, data of **1a**–**1g** are displayed.

Cell culture. MCF-7 cells were obtained from the American Type Culture Collection (ATCC). The cells were maintained with growth medium [Dulbecco's Modified Eagle's Medium (DMEM)-low glucose (D6046, Sigma) supplemented with 10% heat-inactivated fetal bovine serum, penicillin G (100 units/mL), and streptomycin (100 μg/mL)] under atmosphere of 5% CO_2 at 37 °C. The growth medium was refreshed every 2 or 3 d to reach 70–90% cell confluence.
MCF-10A cells were obtained from ATCC. The cells were maintained with mammary epithelial cell growth medium [MEBM (CC3151, Lonza) supplemented with MEGM SingleQuots (CC-4136, Lonza), cholera toxin (100 ng/mL, 030-20621, FUJIFILM Wako Pure Chemical), penicillin G (100 units/mL), and streptomycin (100 μg/mL)] under atmosphere of 5% CO_2 at 37 °C. The growth medium was refreshed every 2 d to reach 70–90% cell confluence.

Mitochondrial ATP production assay. Various concentrations of compounds in DMSO were prepared by serial dilutions. MCF-7 cells were suspended in reaction buffer [5 mM KH_2PO_4/K_2HPO_4 (pH 7.4), 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, 3 mM $MgCl_2$, 5 mM disodium succinate, 2.5 mM glucose, 2 U/mL hexokinase (11426362001, Roche), 2 U/mL glucose-6-phosphate dehydrogenase (9001-40-5, Fujifilm Wako Pure Chemical), 0.25 mM nicotinamide adenine dinucleotide phosphate ($NADP^+$), 25 μM P1,P5-di(adenosine-5')pentaphosphate peptasodium salt (Ap5A), 3 μM rotenone, 0.006% DMSO] at a concentration of 2.5×10^5 cells/mL, and kept on ice. Aliquots of the DMSO (1.5 μL) containing the compounds were added to each well of a clear flat-bottom 96-well plate (TR5003, TrueLine). For calculating 100% or 0% control, DMSO (1.5 μL) or a solution of oligomycin A in DMSO (1 mM, 1.5 μL) were added to each well of the plate. A suspension of digitonin (10 mg/mL, 1.5 μL/well) was added to all the wells of the plate. The suspension of the cells in the reaction buffer (300 μL/well) was added to all the wells of the plate. The plate was shaken at 37 °C for 5 min, and then a solution of ADP in H_2O (100 mM, 1.5 μL/well) was added to all the wells of the plate. The UV absorbance of each well at 340 nm was measured for 1 h (every 1 min) at 37 °C with shaking by using the kinetic protocol of Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific). The rate of change of UV absorbance was calculated from the slope of linear approximation. The linear approximation was performed in the time range where the line showed $r^2 > 0.99$. All the experiments were conducted in triplicate. Each slope was plotted against the concentration of the compound, and then sigmoidal curve fittings were performed on Prism 4 (GraphPad Software, Figure S23). EC_{50} values of **1a**–**1g** were determined by three independent experiments. The EC_{50} values were statistically compared with that of **1a** using Dunnett's test.

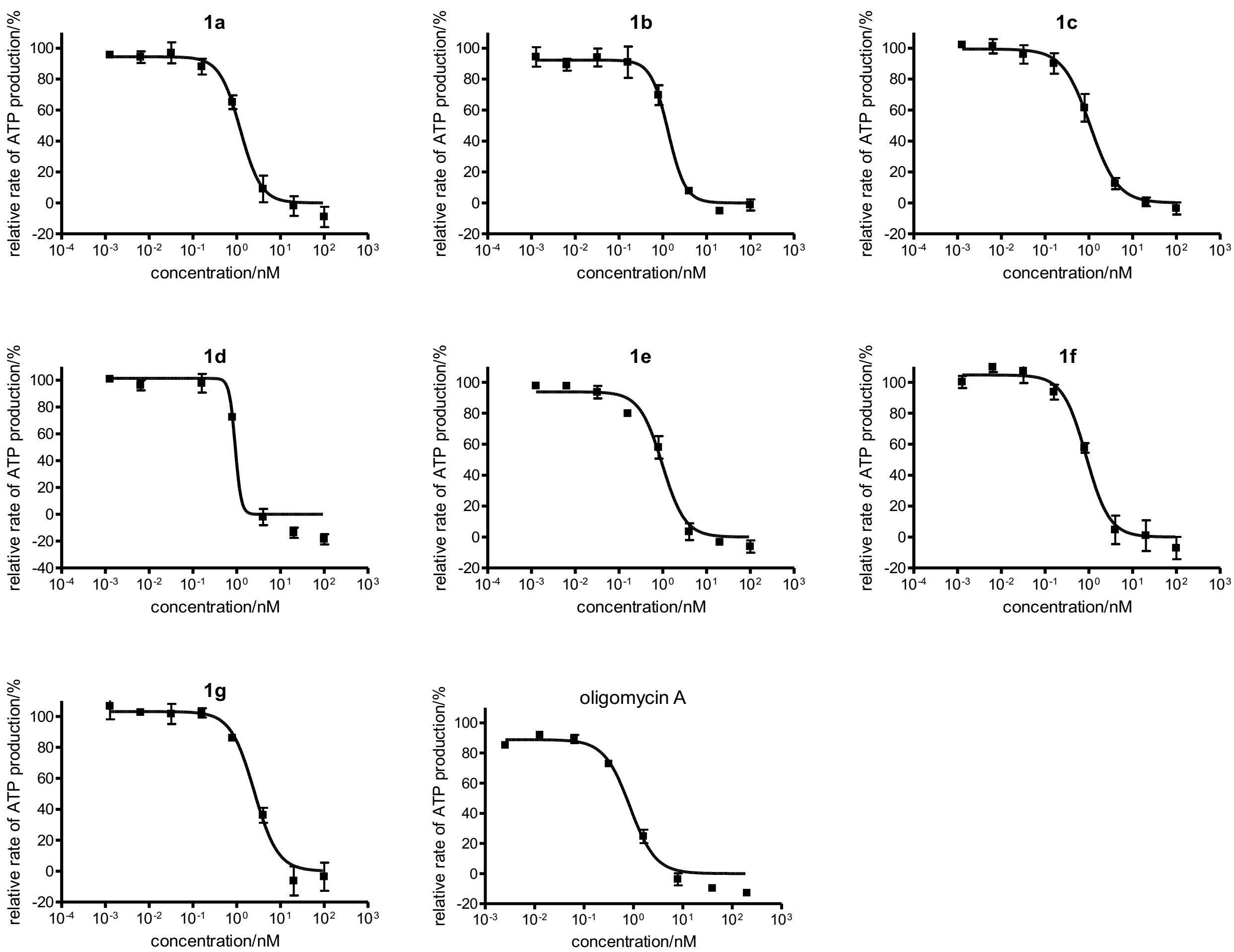


Figure S23. Representative concentration-response curves of **1a**–**1g** for ATP production rate against MCF-7 cells. Oligomycin A was used as a positive control. Each plot is displayed as mean ± SD of three replicates.

Sulforhodamine B assay. Growth inhibitory activities of the tested compounds were evaluated according to the literature.[S5] Cell growth (%) was defined as follows:

$$\text{cell growth (\%)} = \frac{FL(sample) - FL(day0)}{FL(control) - FL(day0)} \times 100 \qquad \{FL(sample) \geq FL(day0)\}$$

$$\text{cell growth (\%)} = \frac{FL(sample) - FL(day0)}{FL(day0)} \times 100 \qquad \{FL(day0) > FL(sample)\}$$

FL = mean of the fluorescence intensity (Ex. 485 nm/Em. 585 nm)
*day*0 = time of addition of the tested compound as serial dilutions
control = control wells treated by vehicle (DMSO)

Various concentrations of compounds in the growth medium containing 2% DMSO were prepared by serial dilutions. MCF-7 cells or MCF-10A cells were cultured in 6 cm cell culture dishes filled with the growth medium and harvested by trypsinization at 37 °C for 5–10 min. The collected cells were resuspended into the growth medium at 1.25×10^5 cells/mL (MCF-7 cells) or 2.00×10^5 cells/mL (MCF-10A cells). The cell suspension (100 μL/well) was seeded into the black polystyrene flat-bottom 96-well plates (sample-plates, 655090, Greiner bio-one). For calculating *FL*(*day*0), the same cell suspension (100 μL/well) was seeded into an independent 96-well plate (*day*0-plate). The sample-plate and *day*0-plate were incubated at 37 °C under atmosphere of 5% CO_2 for 24 h. Aliquots of the former medium (100 μL) containing compounds were added to each well of the sample-plate. The growth medium containing 2% DMSO (100 μL) was added to the wells for calculating *FL*(*control*) of the sample-plate and the wells for calculating *FL*(*day*0) of the *day*0-plate. To the cells in *day*0-plate was added an ice-cold solution of 30 w/v% trichloroacetic acid (TCA) in H_2O (100 μL/well). The *day*0-plate was incubated at 4 °C for 60 min, washed with H_2O (× 4), dried, and stored at room temperature. The sample-plate was incubated at 37 °C under atmosphere of 5% CO_2 for 48 h. To the cells in the sample-plate was added an ice-cold solution of 30 w/v% TCA in H_2O (100 μL/well). The sample-plate was incubated at 4 °C for 60 min, washed with H_2O (× 4), and dried. To each well of the *day*0-plate and sample-plate was added a solution of sulforhodamine B in AcOH/H_2O (1/99, 570 μg/mL, 100 μL/well). The fixed cells were stained at room temperature for 30 min in the dark. The cells were washed with AcOH/H_2O (1/99, × 4) and dried. To the stained cells was added a solution of 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) in H_2O (10 mM, 200 μL/well). The plates were vortexed at room temperature for 10 min. The fluorescence (Ex. 485 nm/Em. 585 nm) of each well was measured on Spectra Max Gemini EM microplate reader (Molecular Devices). Growth inhibitory activity of each compound was evaluated as GI_{50} (nM) by means of three replicates. Sigmoidal curve fittings were performed on Prism 4 (GraphPad Software, Figure S24 and Figure S25). GI_{50} values of **1a**–**1g** were determined by three independent experiments. The GI_{50} values were statistically compared with that of **1a** using Dunnett's test.

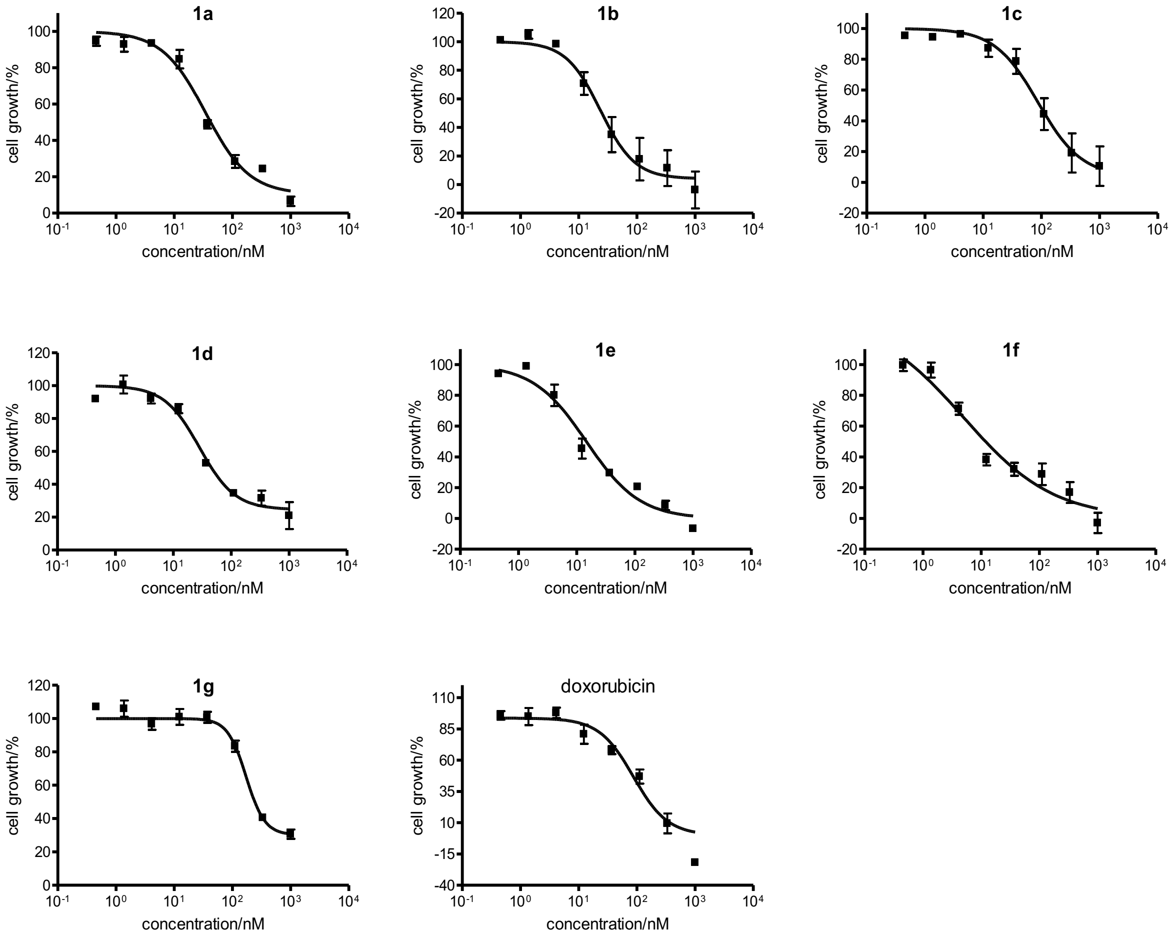


Figure S24. Representative concentration-response curves of **1a**–**1g** against MCF-7 cells. Doxorubicin was used as a positive control. The cells were incubated for 2 d in the presence of a peptide, and the cell growth (%) was evaluated. Each plot is displayed as mean ± SD of three replicates.

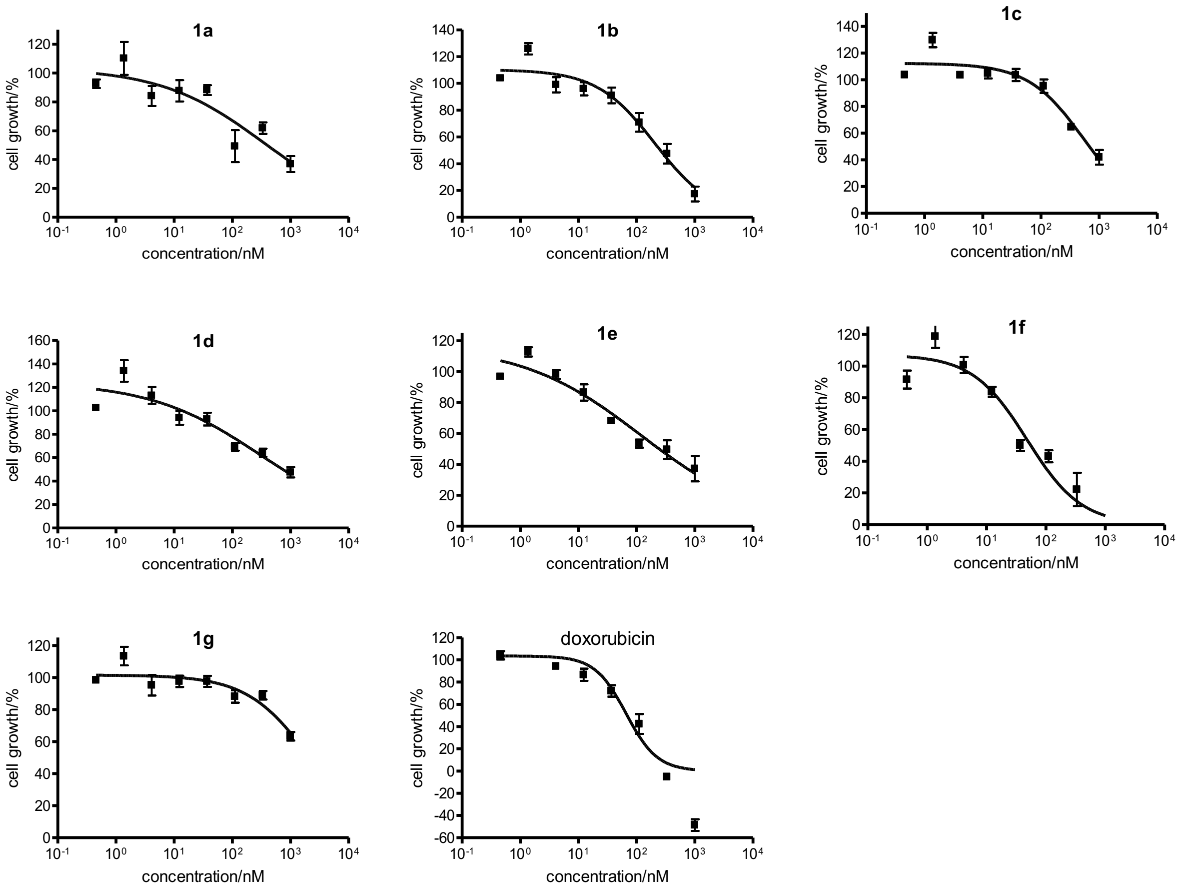


Figure S25. Representative concentration-response curves of **1a–1g** against MCF-10A cells. Doxorubicin was used as a positive control. The cells were incubated for 2 d in the presence of a peptide, and the cell growth (%) was evaluated. Each plot is displayed as mean ± SD of three replicates.

Evaluation of proteolystic stability against Pronase. Each compound (**1a** or **1f**, 15.0 nmol) was incubated with Pronase (a mixture of multiple proteases from *Streptomyces griseus*, 4.00 mg) in phosphate buffered saline (200 μL) at 37 °C. After the incubation for 0, 1, 2, and 4 d, an aliquot (30.0 μL) of the reaction mixture was collected, and mixed with MeOH containing 0.05% TFA (30.0 μL). The concentration of the remaining compound in the reaction mixture was determined by UHPLC analysis [column: Inertsil C8-3 2.1 × 150 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 74/26, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC]. Under these conditions, **1a** and **1f** were not degraded within 4 d (Figure S26).

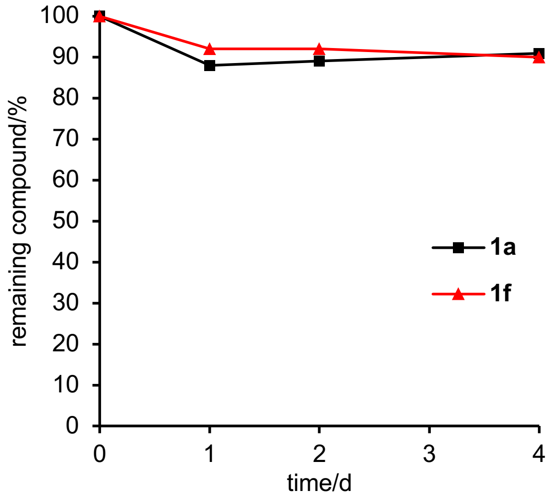
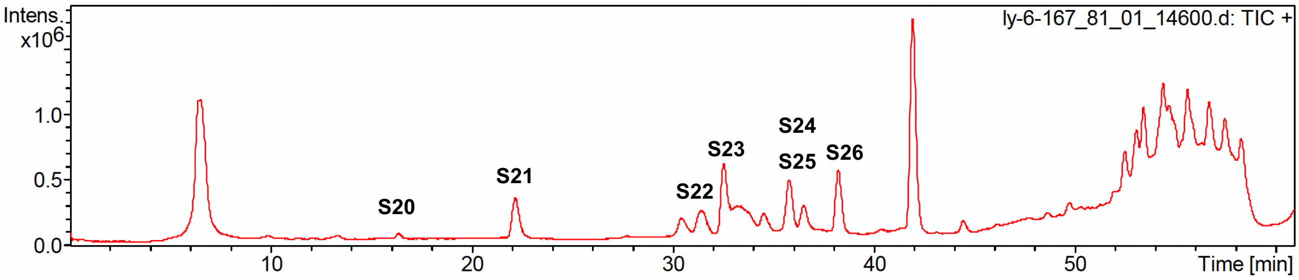
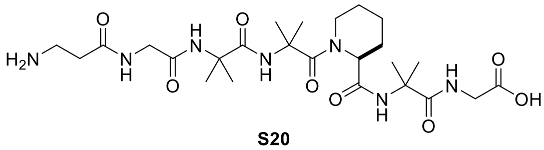


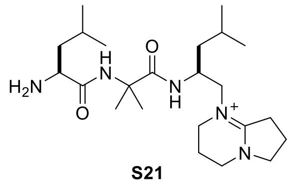
Figure S26. Proteolytic stability of **1a** and **1f** (71.0 μM) against Pronase (20.0 mg/mL). The amount of remaining compound (%) was analyzed during 4-d incubation.

Evaluation of proteolytic stability against papain. Each compound (**1a**, **1f**, or benzoyl-L-arginine *p*-nitroanilide, 15.0 nmol) was incubated with papain (4.00 mg) in 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) buffer (pH 6.8, 0.05 M, 200 μL) containing 2-mercaptoethanol (20.0 mM) and ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid (EDTA, 500 μM) at 37 °C. After the incubation for 0, 1, 2, 4, 8, and 24 h (**1a** and **1f**) or the incubation for 0, 5, 15, 30, and 60 min (benzoyl-L-arginine *p*-nitroanilide), an aliquot (30.0 μL) of the reaction mixture was collected, and mixed with MeOH containing 0.05% TFA (30.0 μL). The concentration of the remaining compound in the reaction mixture was determined by UHPLC analysis [column: Inertsil C8-3 2.1 × 150 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 74/26 for **1a** and **1f**, 50/50 for benzoyl-L-arginine *p*-nitroanilide, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm for **1a** and **1f**, 254 nm for benzoyl-L-arginine *p*-nitroanilide), temperature: 40 ºC].

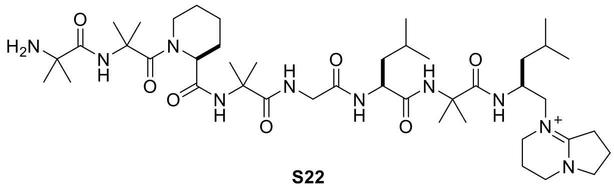




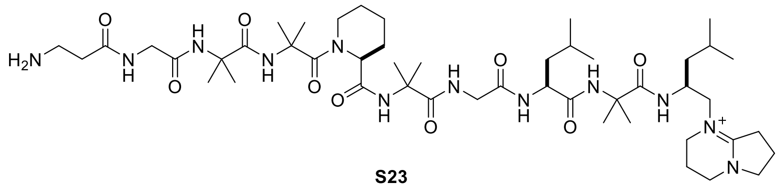
S20



S21



S22



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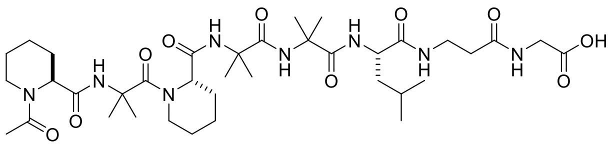

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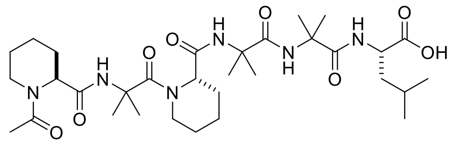
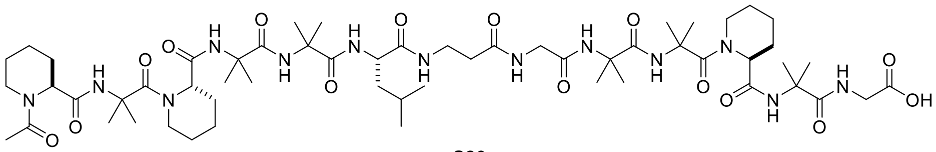


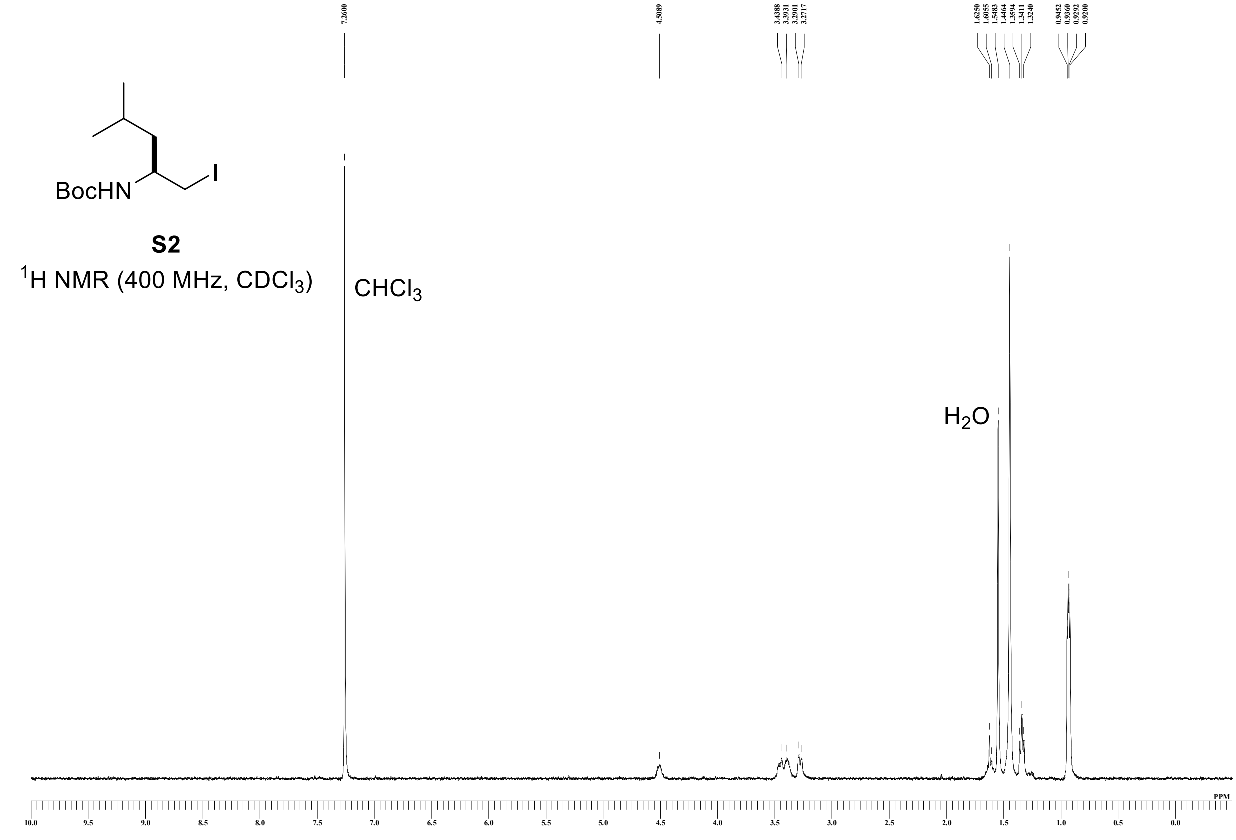
S25



S26

Figure S27. Total ion chromatogram of degraded **1a** in the presence of papain. The chromatogram was acquired after the incubation of **1a** with papain for 24 h. The structures of **S20–S26** were tentatively assigned based on mass spectra. Column: Inertsil C8-3 4.6 × 150 mm, eluent A: MeOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, linear gradient A/B = 10/90 to 100/0 over 60 min, flow rate: 1.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

NMR Charts



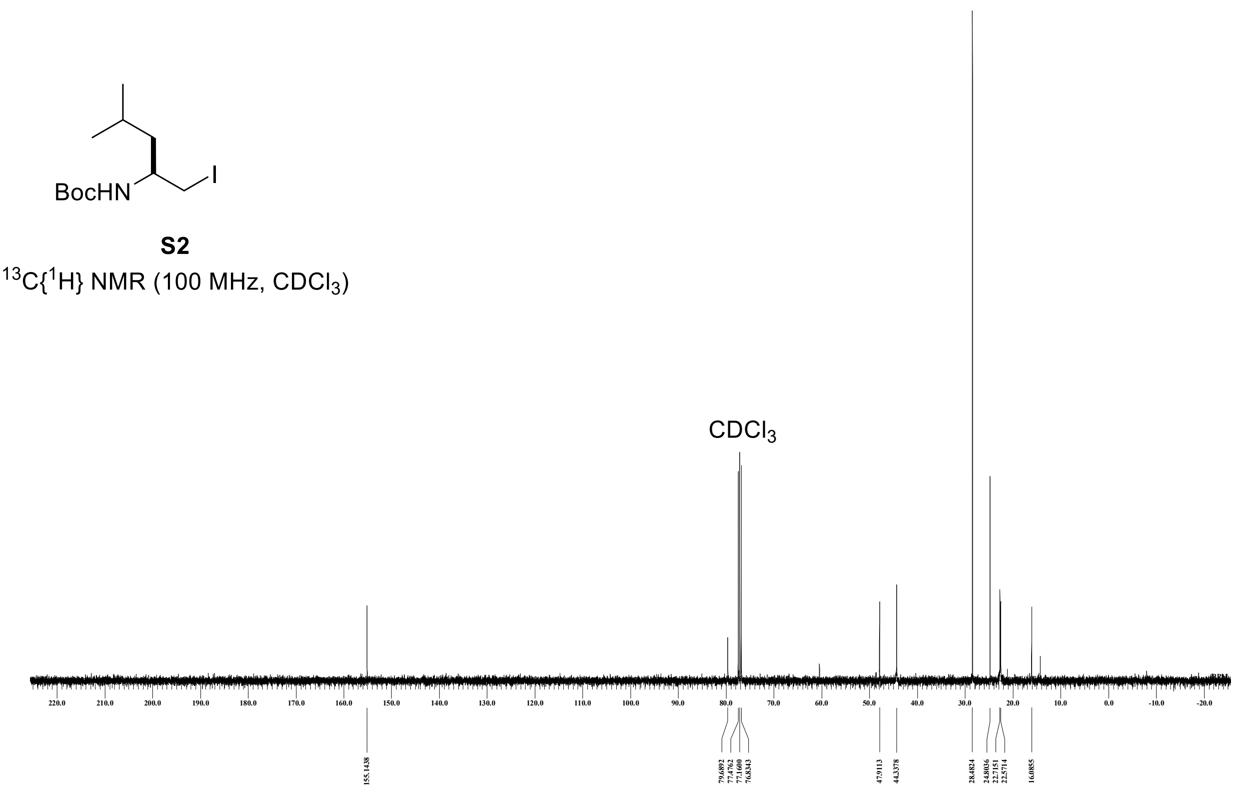
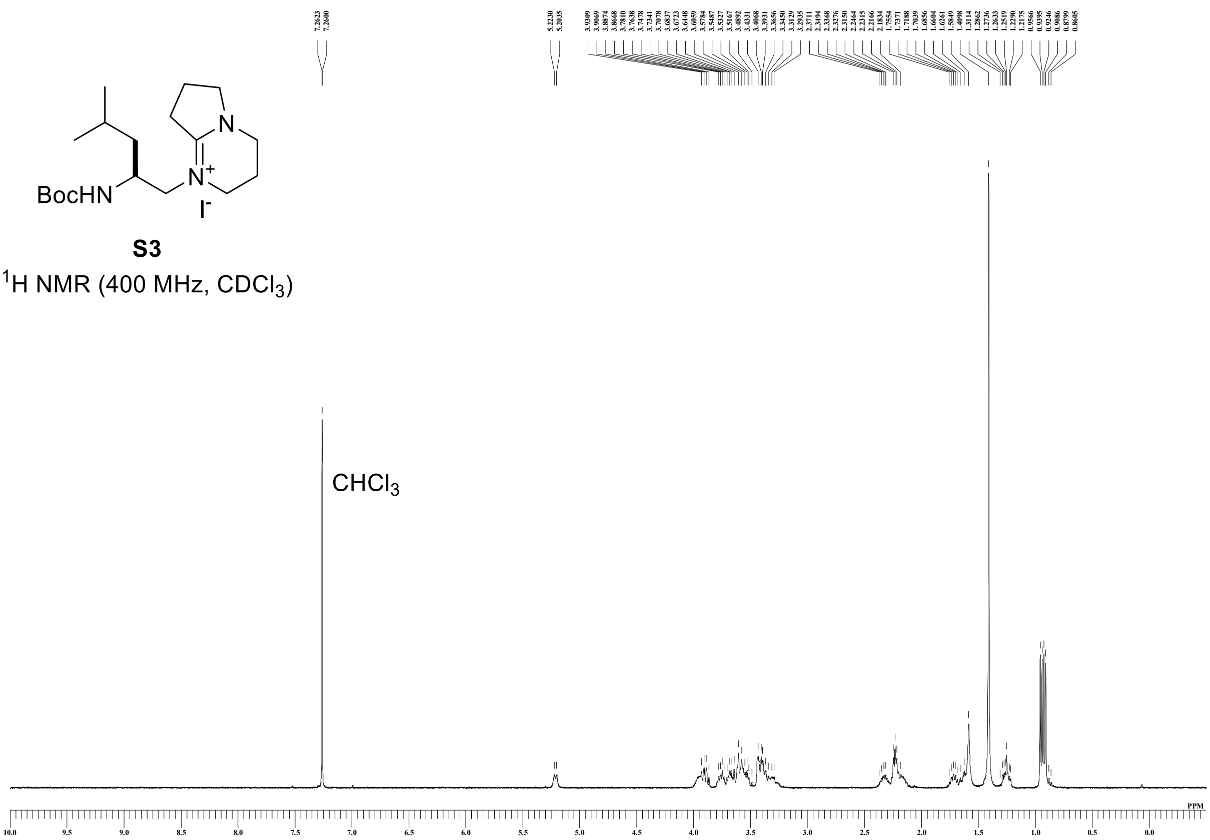


Figure S28. ^{1}H and ^{13}C{^{1}H} NMR spectra of **S2**.



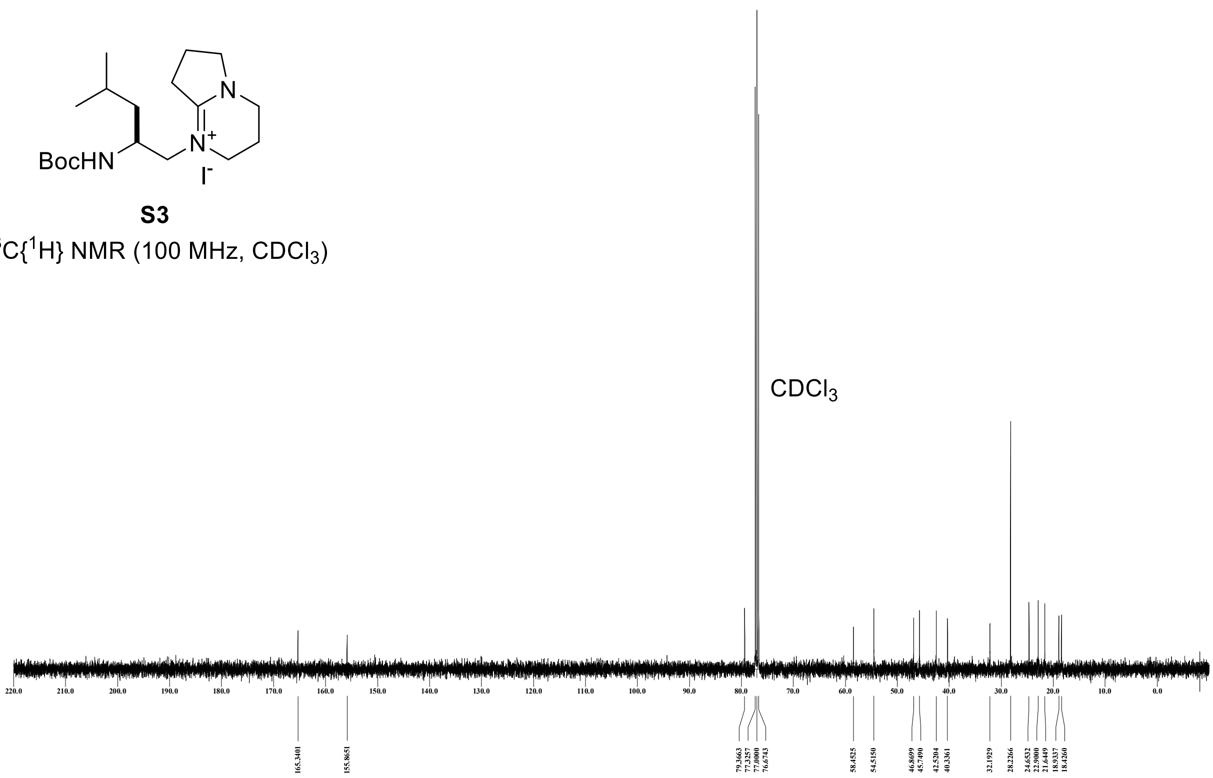
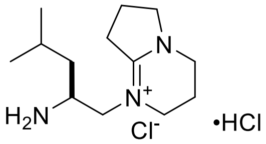
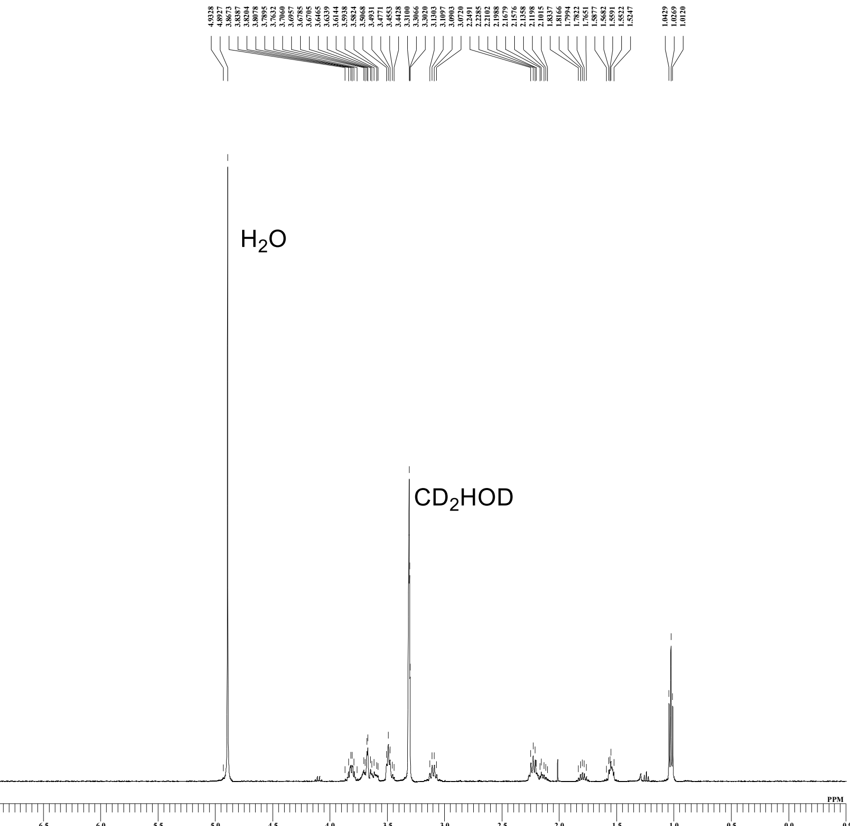


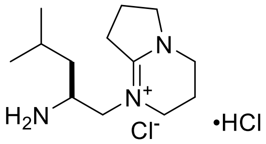
Figure S29. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **S3**.



7

^{1}H NMR (400 MHz, CD_3OD)





7

$^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD)

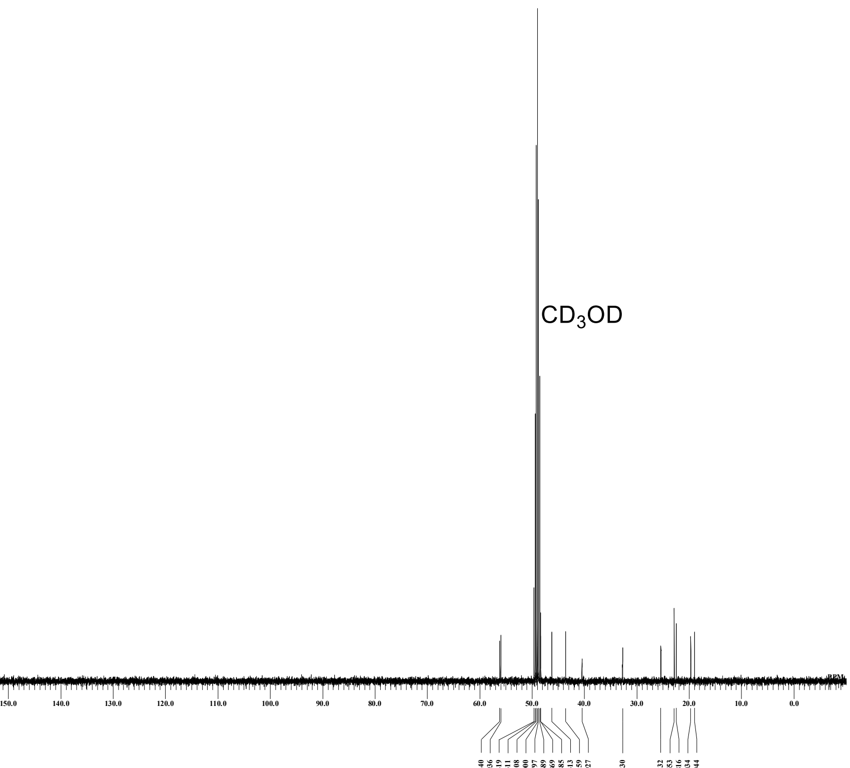
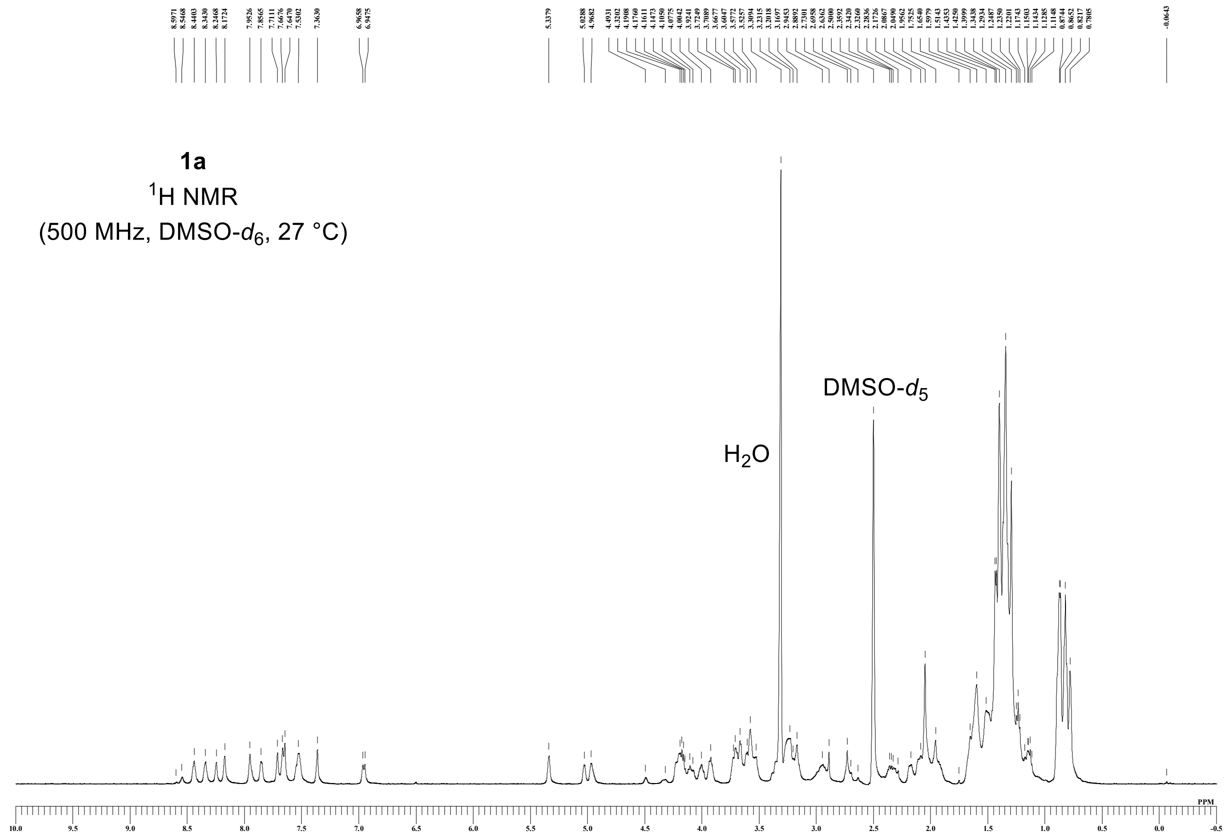


Figure S30. ^{1}H and $^{13}C\{^1H\}$ NMR spectra of **7**.



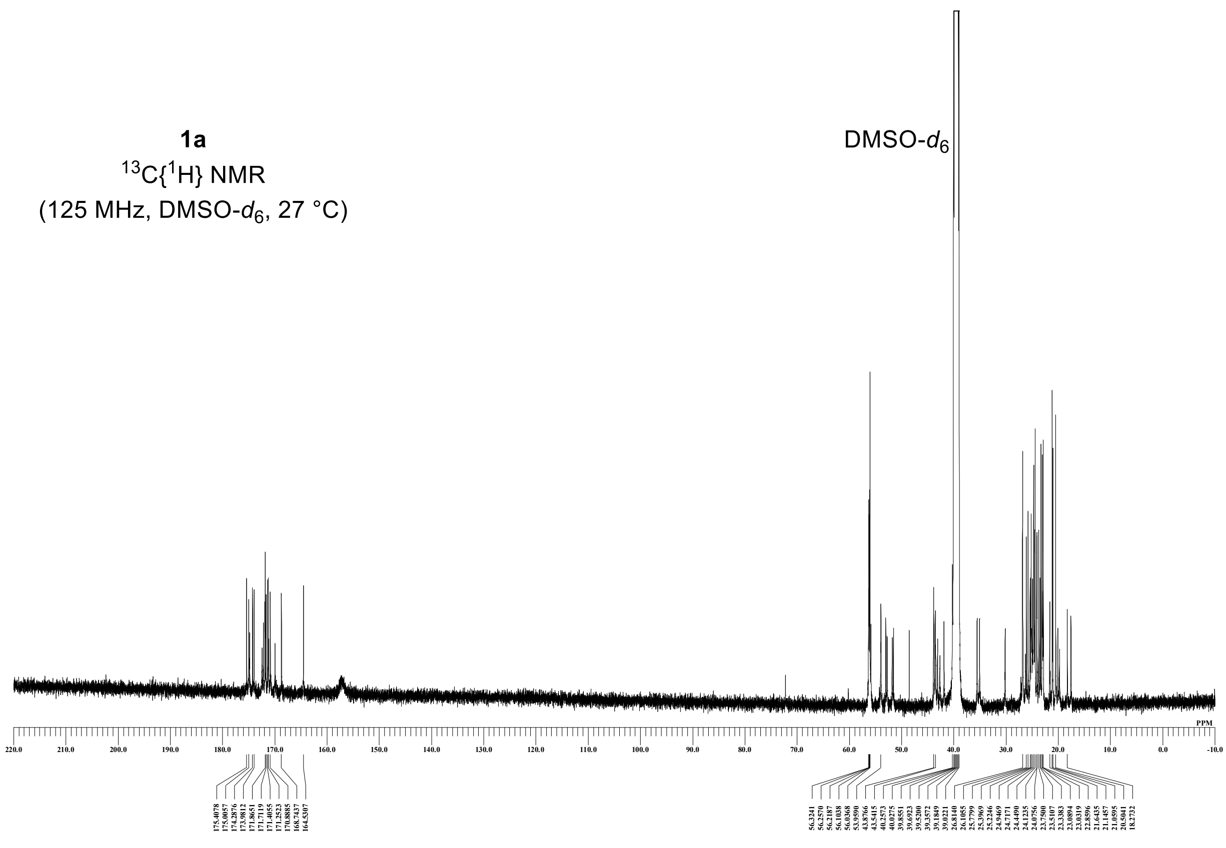
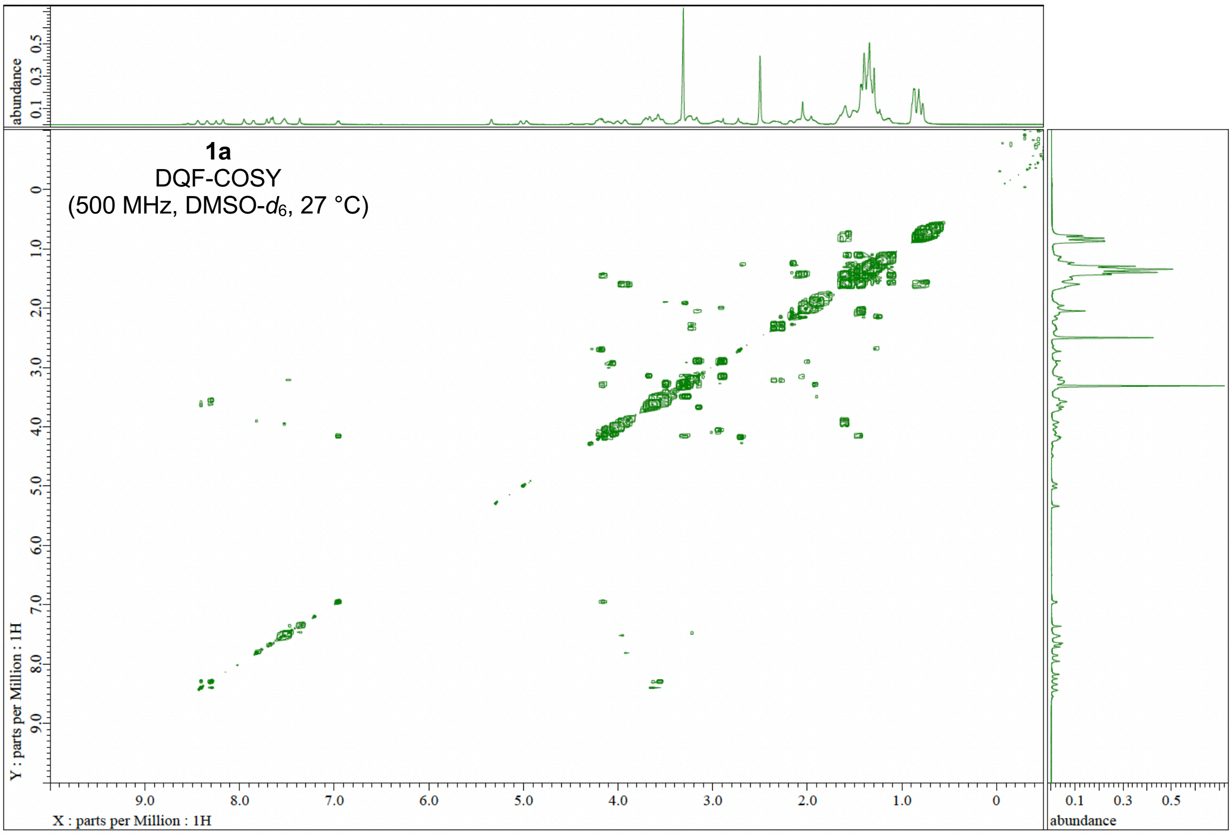


Figure S31. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **1a**. The spectra were obtained in DMSO-d_6 at 27 °C.



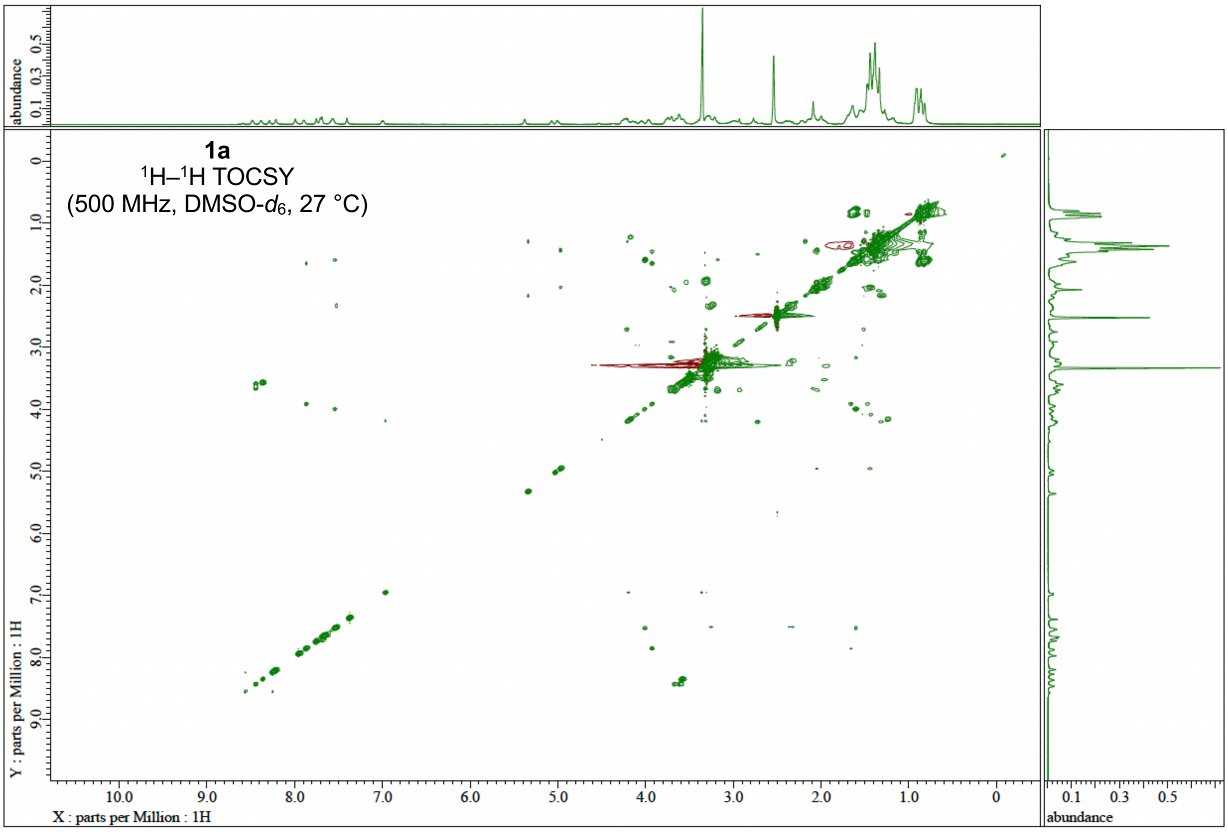
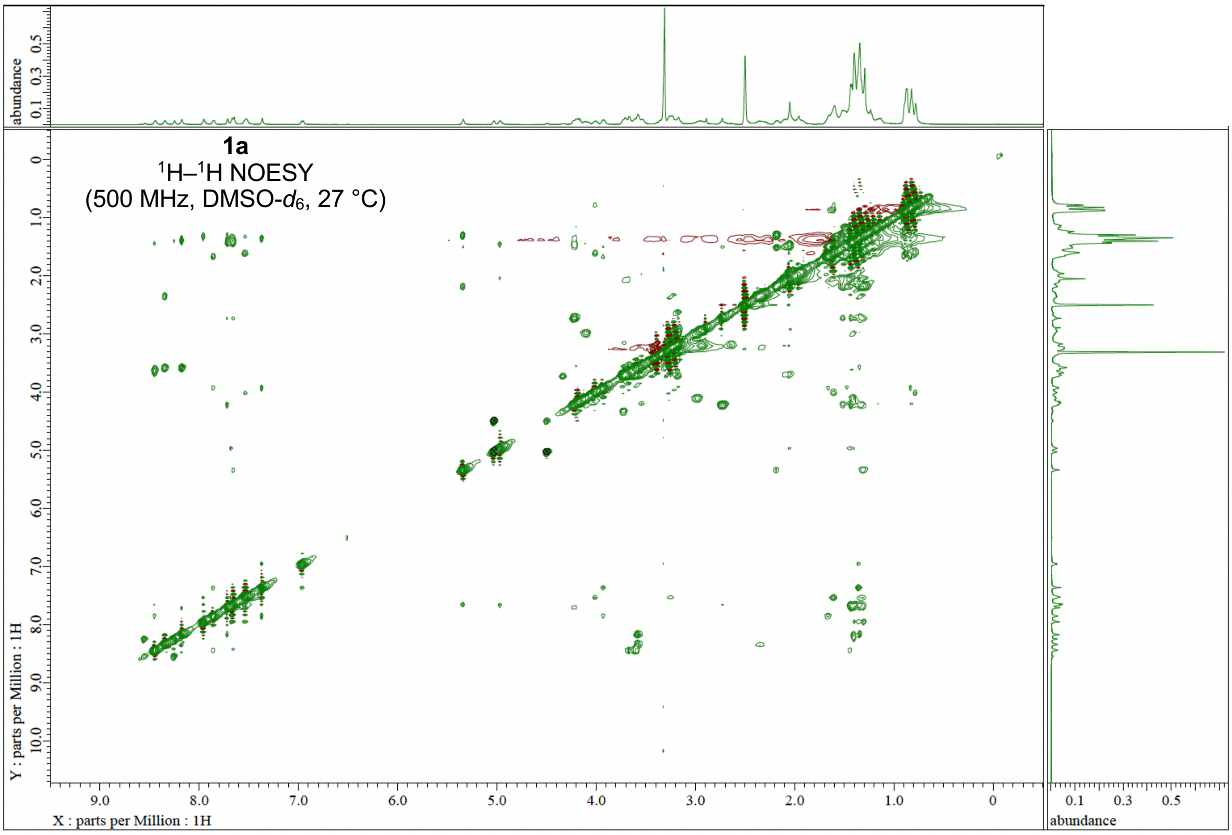


Figure S32. ^{1}H–^{1}H DQF-COSY and ^{1}H–^{1}H TOCSY spectra of **1a**. The spectra were obtained in DMSO-d_6 at 27 °C.



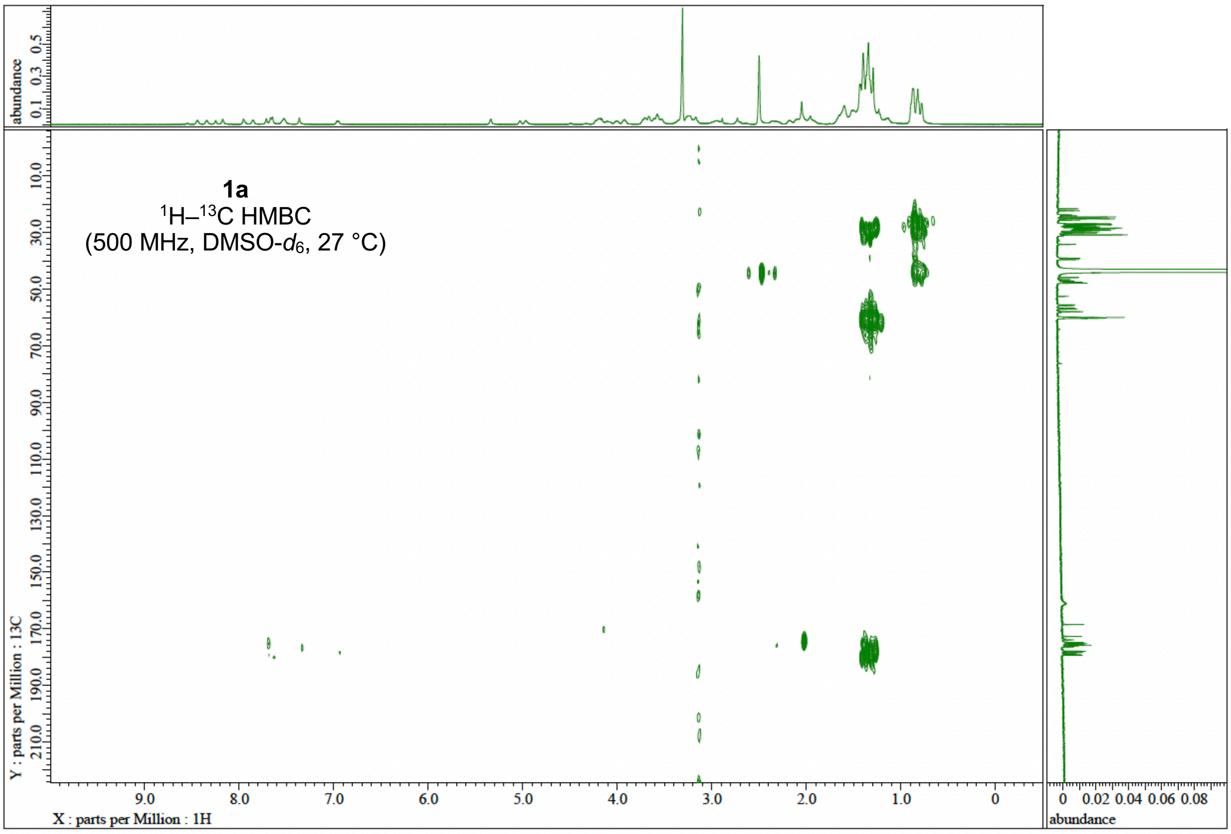


Figure S33. ^{1}H–^{1}H NOESY and ^{1}H–^{13}C HMBC spectra of **1a**. The spectra were obtained in DMSO-d_6 at 27 °C.

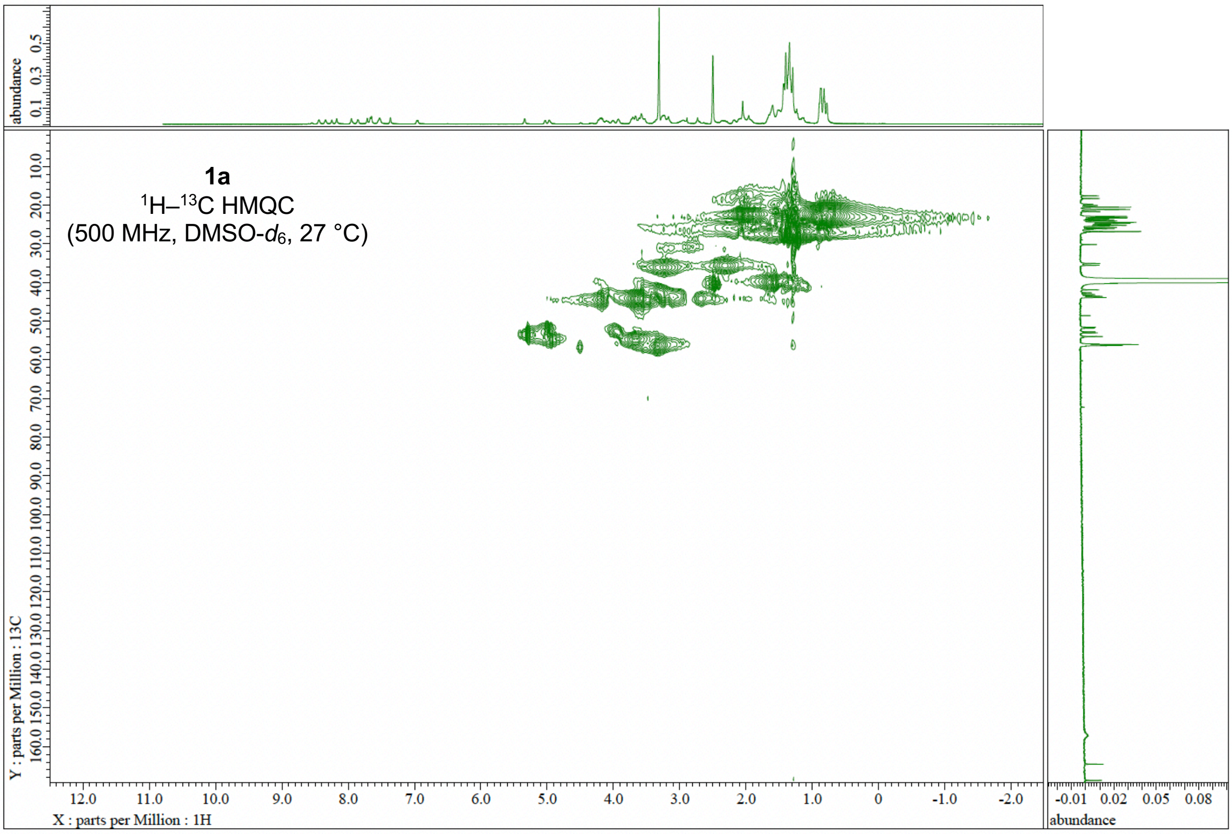


Figure S34. ^{1}H–^{13}C HMQC spectrum of **1a**. The spectrum was obtained in DMSO-d_6 at 27 °C.

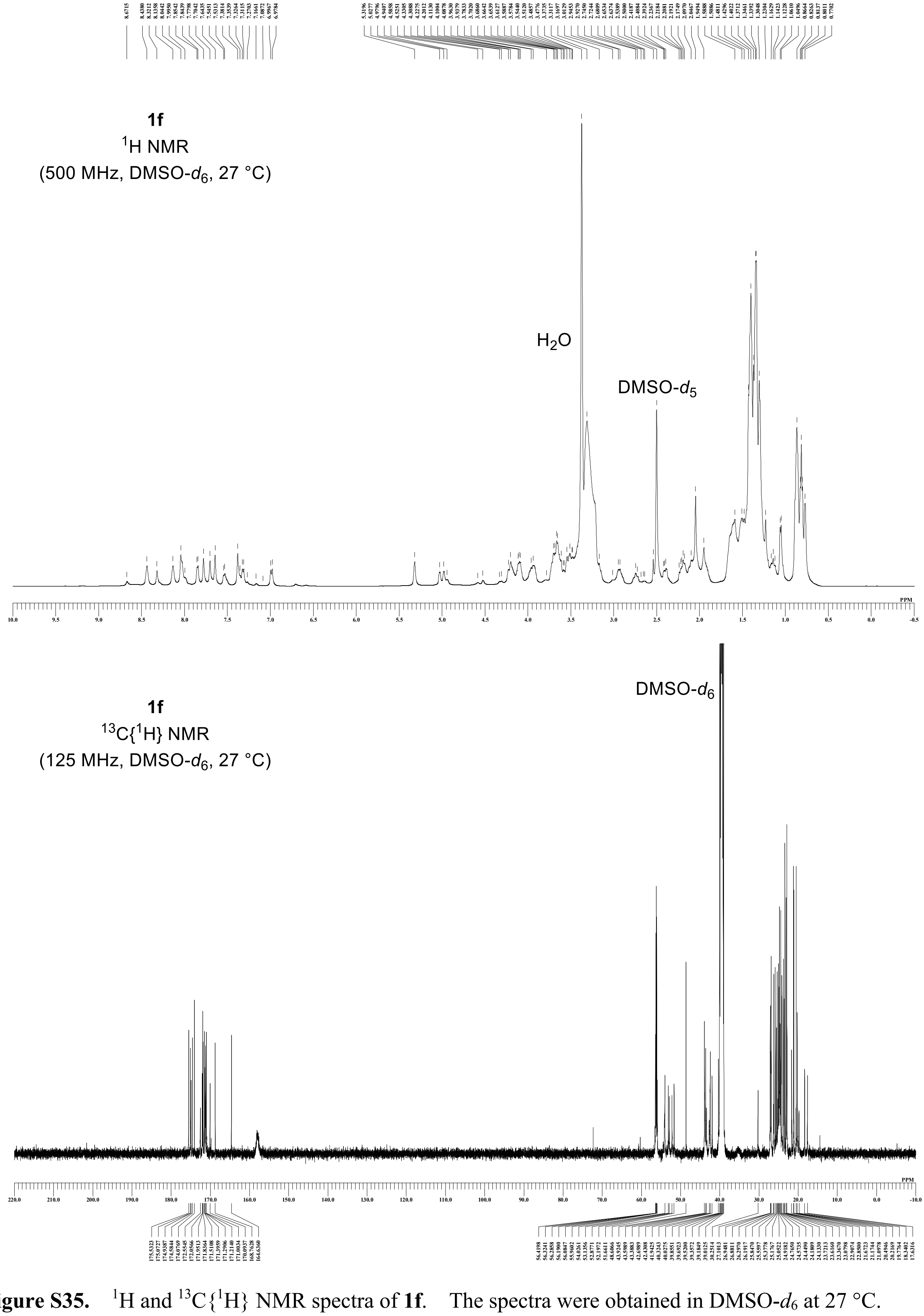


Figure S35. ^{1}H and ^{13}C{^{1}H} NMR spectra of **1f**. The spectra were obtained in DMSO-d_6 at 27 °C.

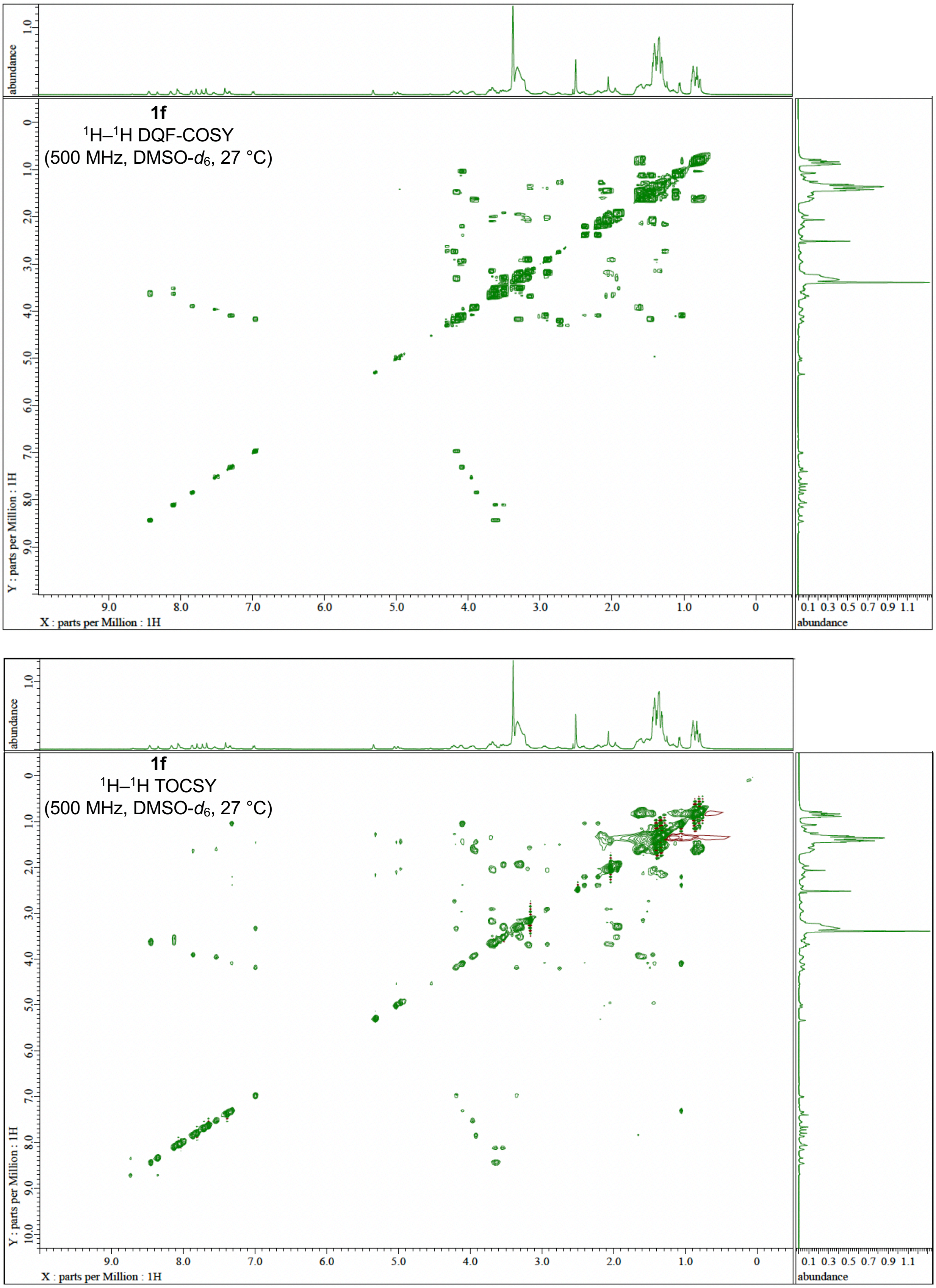
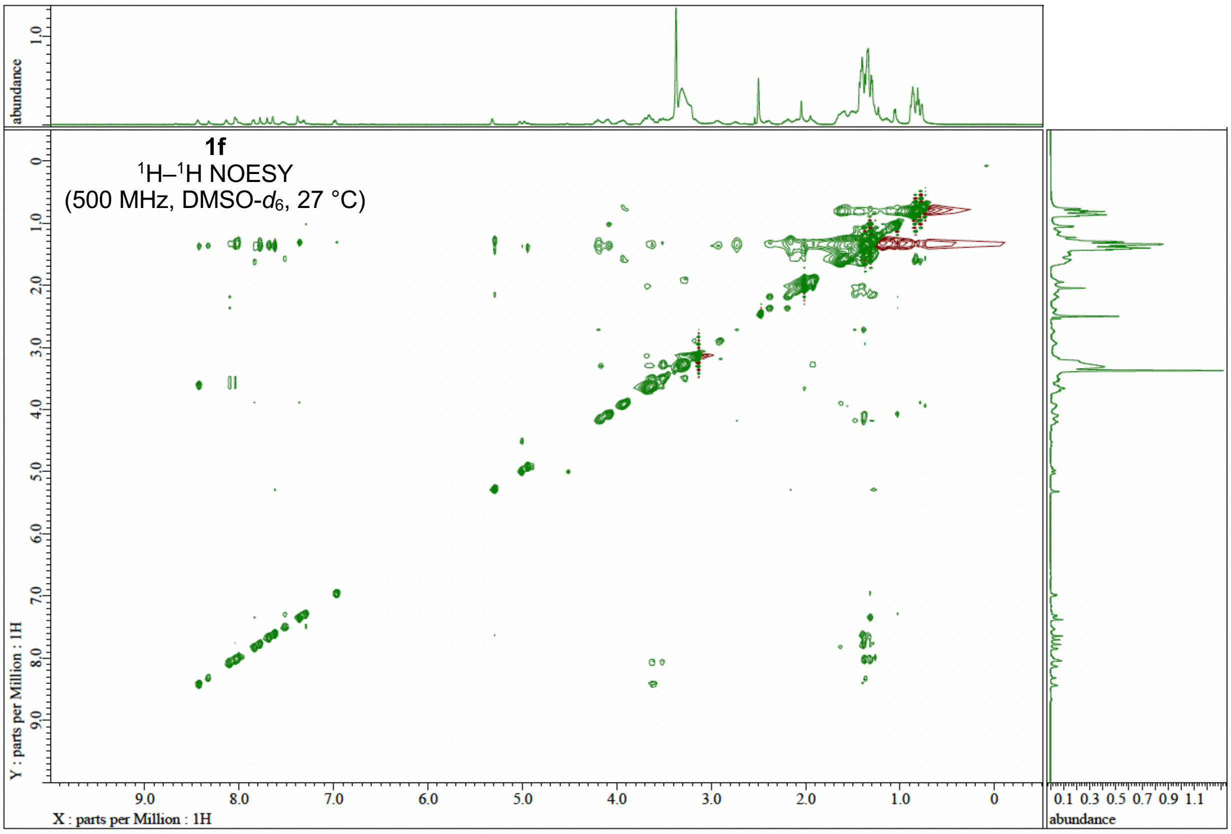


Figure S36. ¹H–¹H DQF-COSY and ¹H–¹H TOCSY spectra of **1f**. The spectra were obtained in DMSO-d_6 at 27 °C.



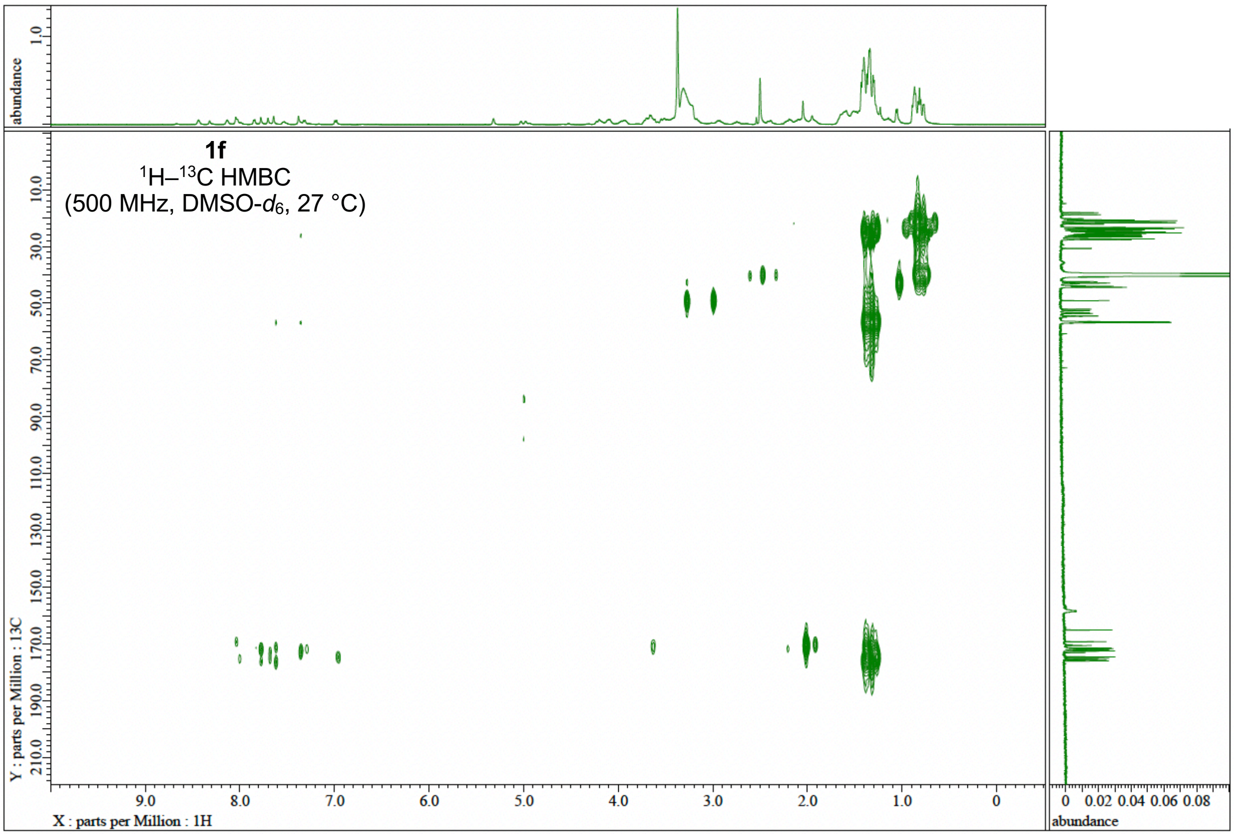


Figure S37. ^{1}H–^{1}H NOESY and ^{1}H–^{13}C HMBC spectra of **1f**. The spectra were obtained in DMSO-d_6 at 27 °C.

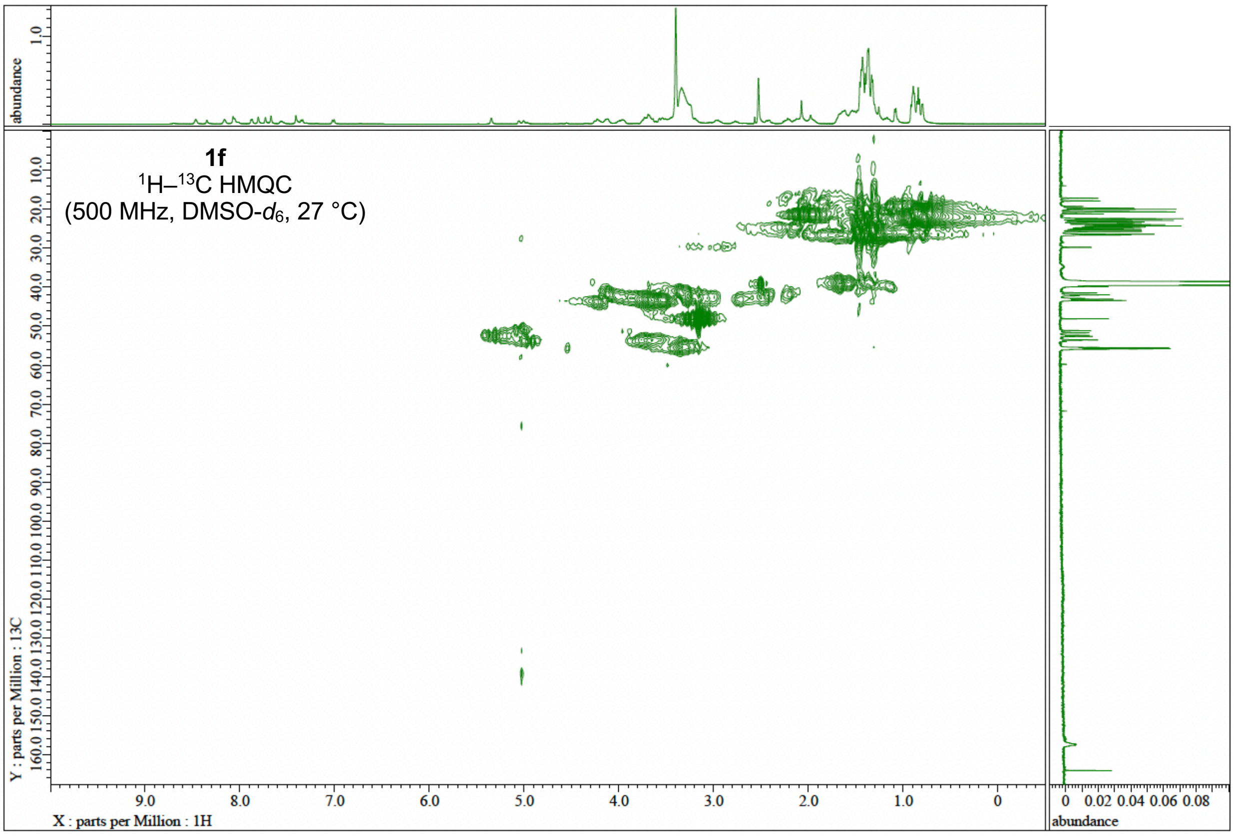
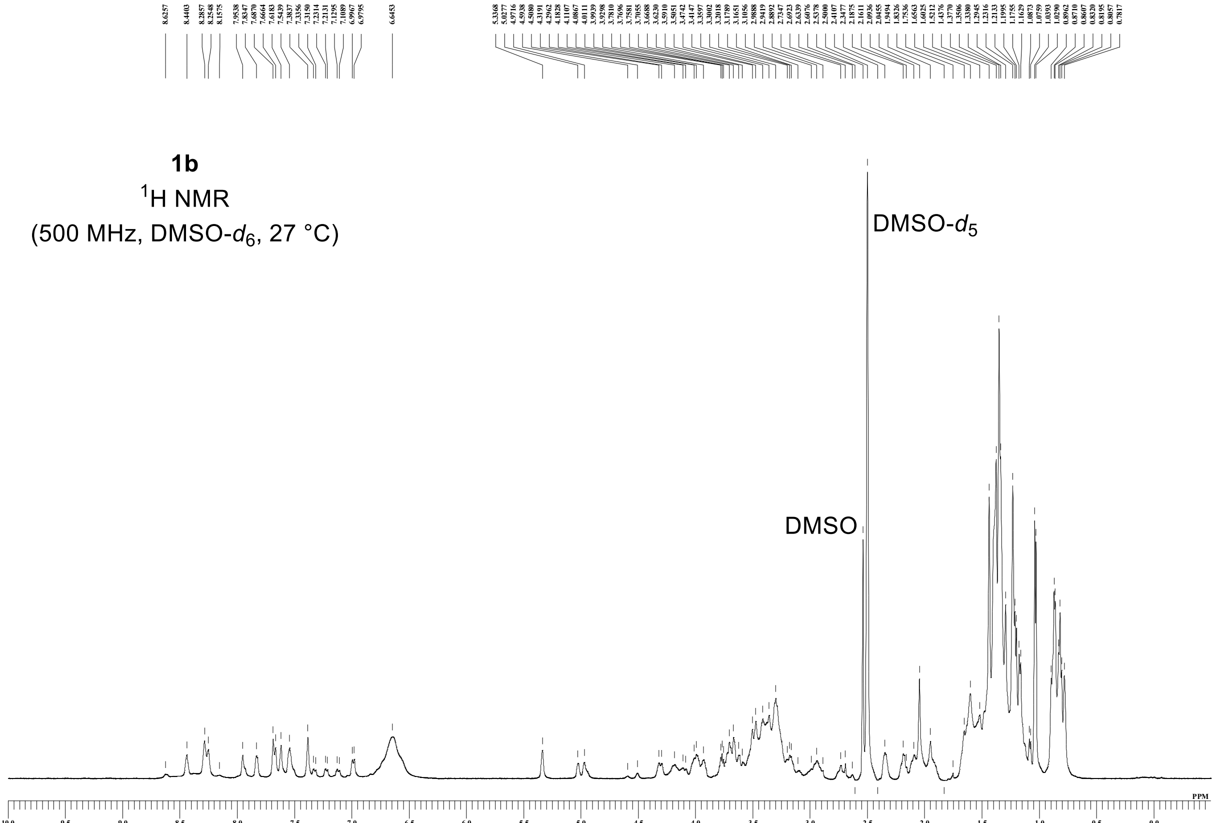


Figure S38. ^1H–^{13}C HMQC spectrum of **1f**. The spectrum was obtained in DMSO-d_6 at 27 °C.



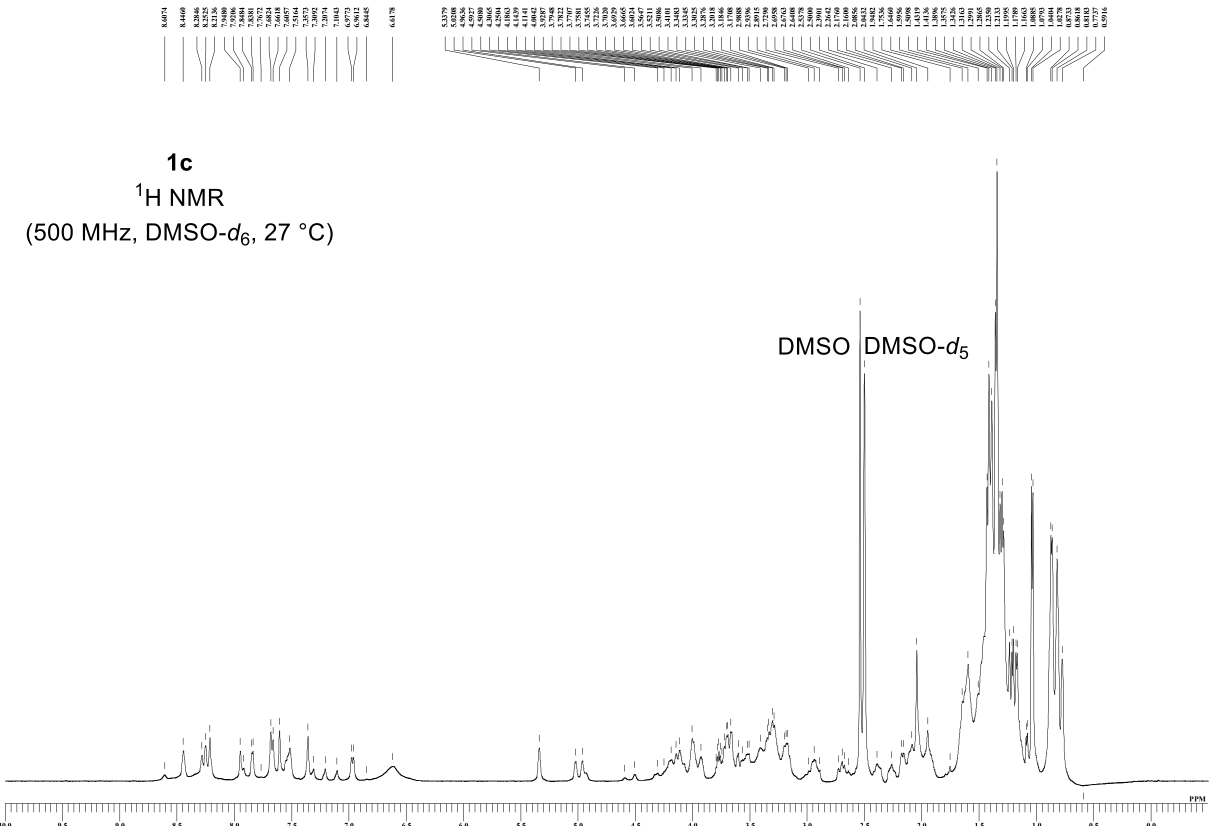
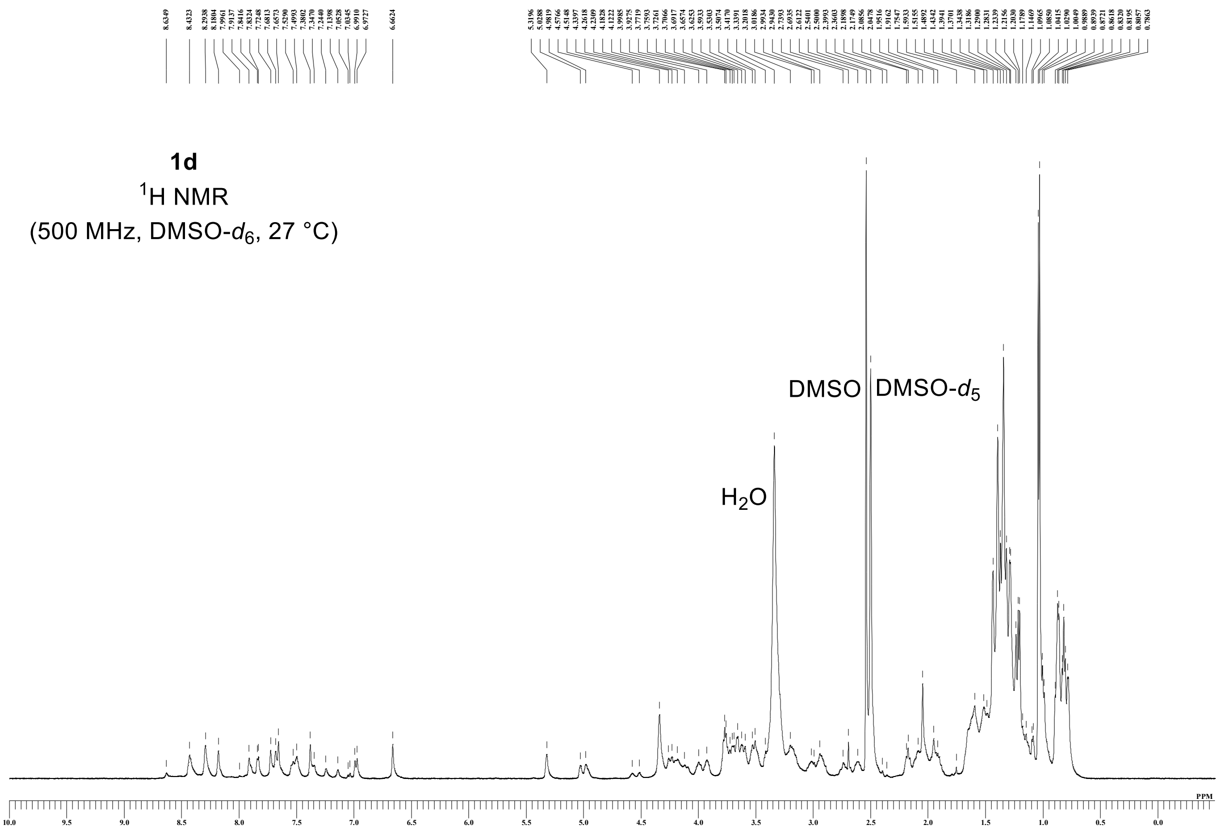


Figure S39. ^{1}H NMR spectra of **1b** and **1c**. The spectra were obtained in DMSO-d_6 at 27 °C.



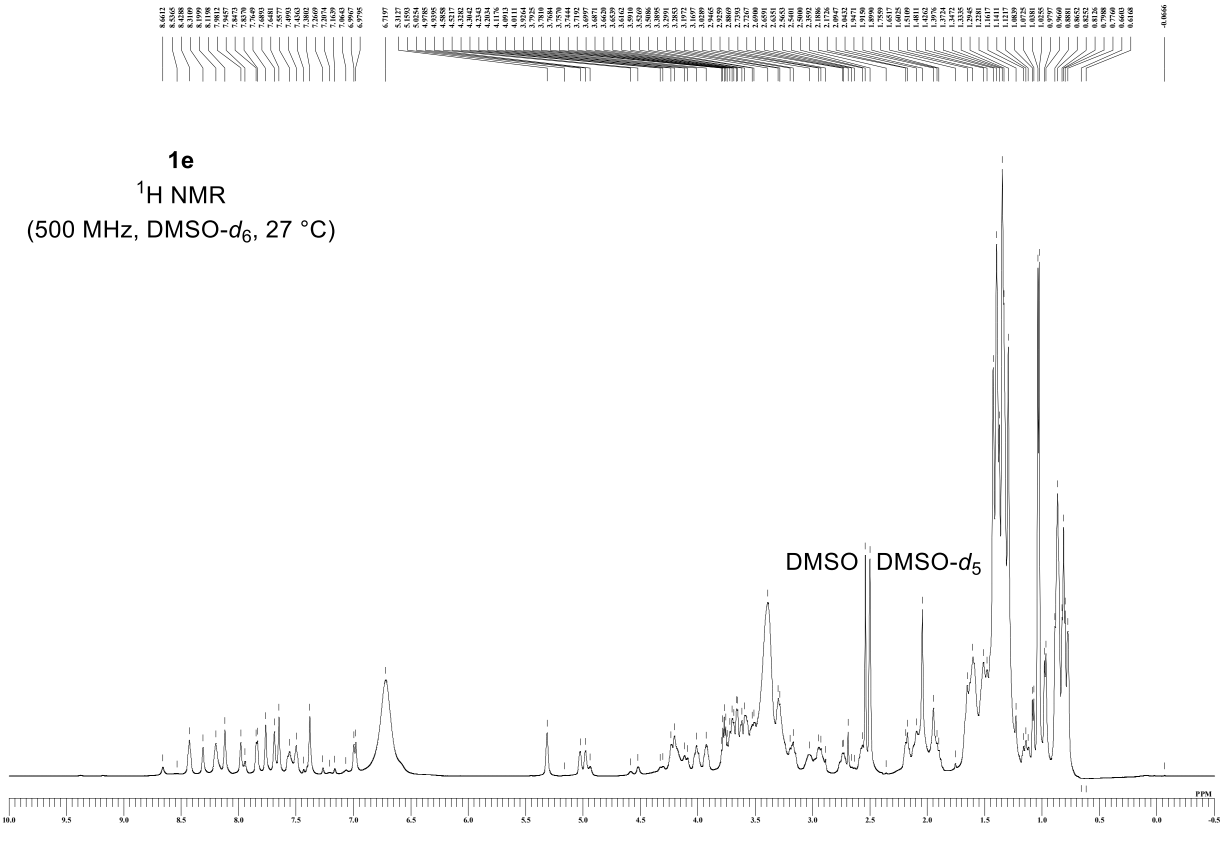


Figure S40. [1]H NMR spectra of **1d** and **1e**. The spectra were obtained in DMSO-d_6 at 27 °C.

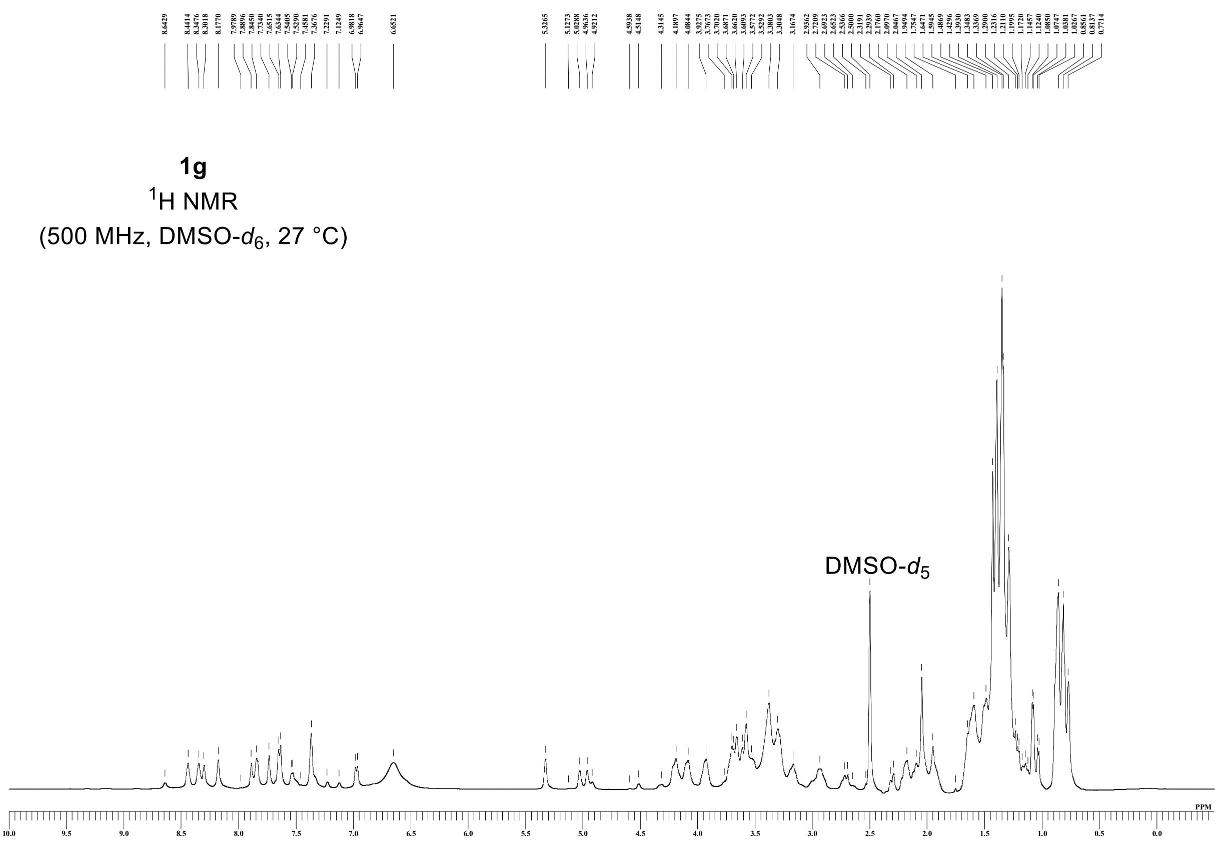


Figure S41. ^{1}H NMR spectrum of **1g**. The spectrum was obtained in DMSO-d_6 at 27 °C.

MS/MS Charts

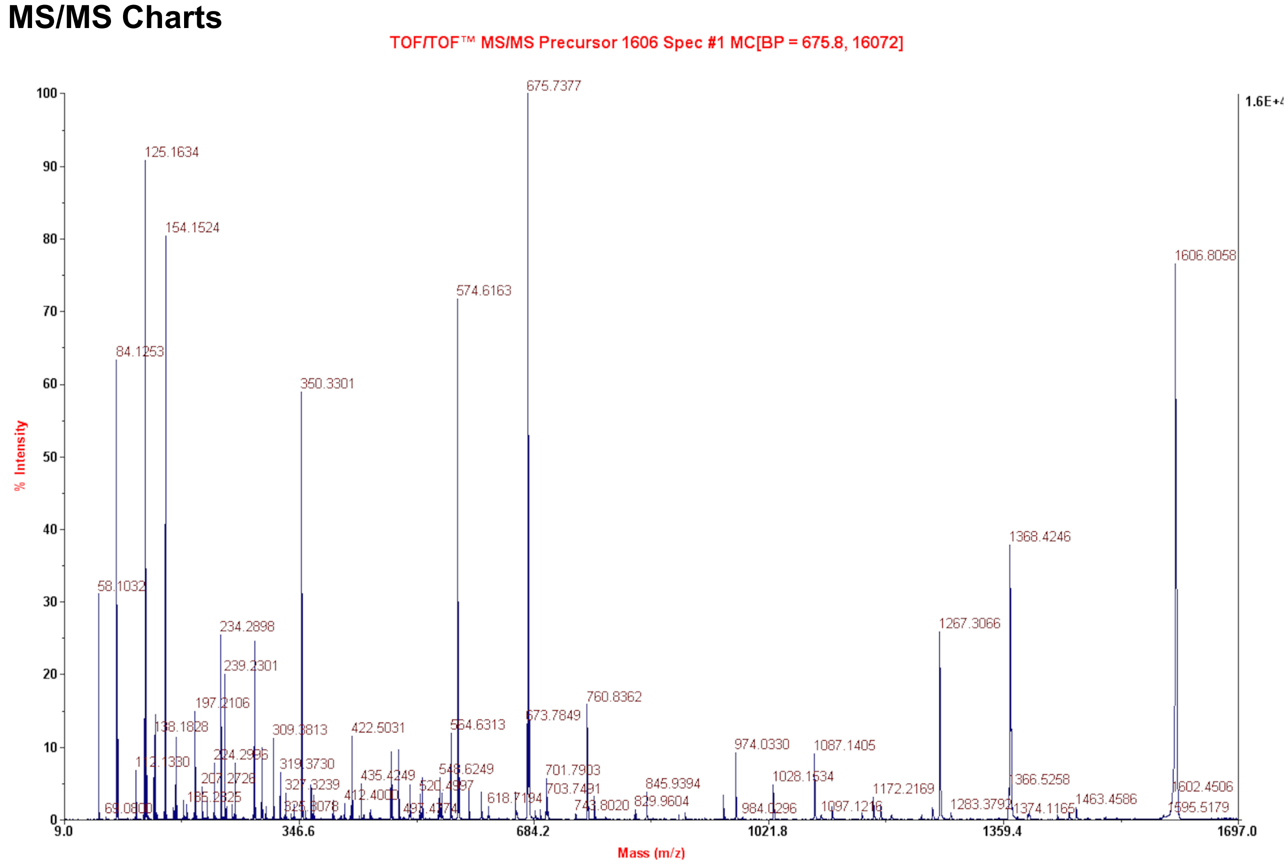


Figure S42. MS/MS spectrum of **1a**.

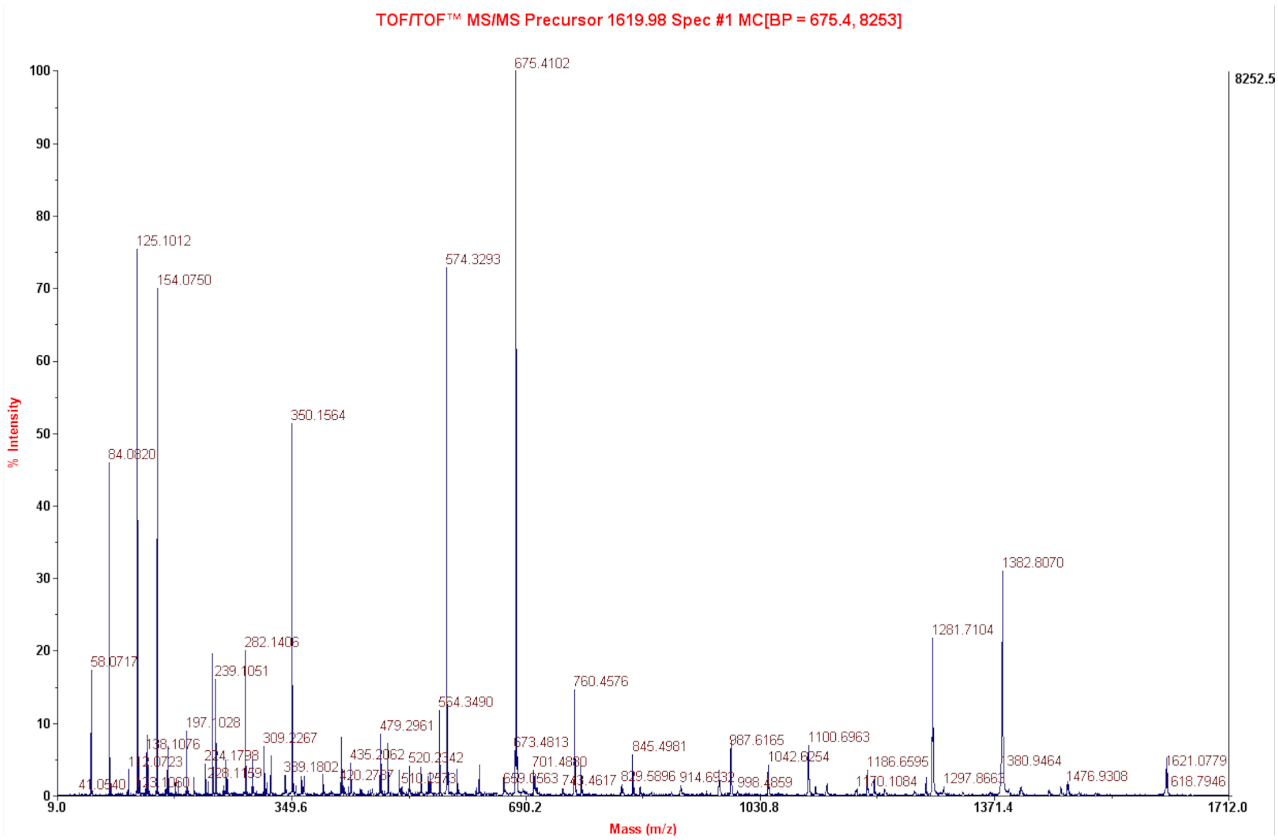


Figure S43. MS/MS spectrum of **1b**.

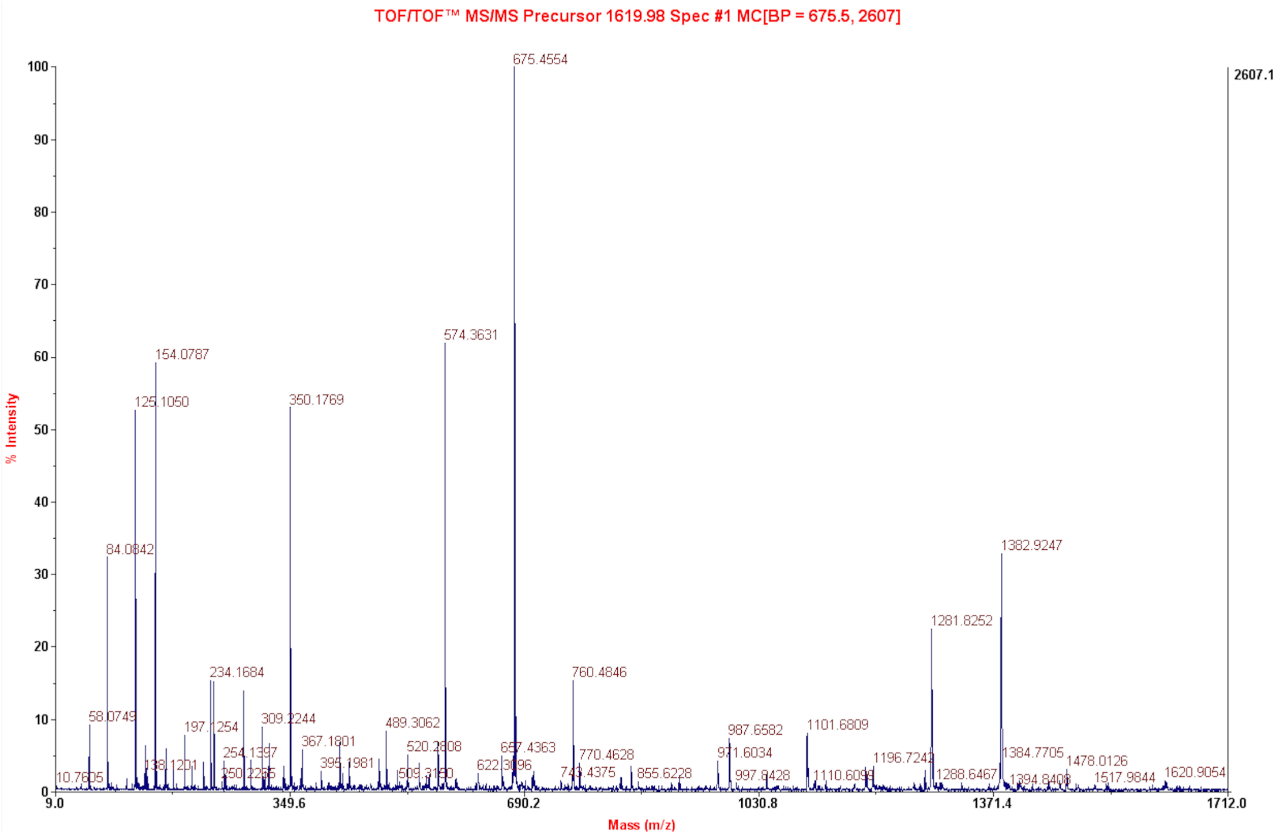


Figure S44. MS/MS spectrum of **1c**.

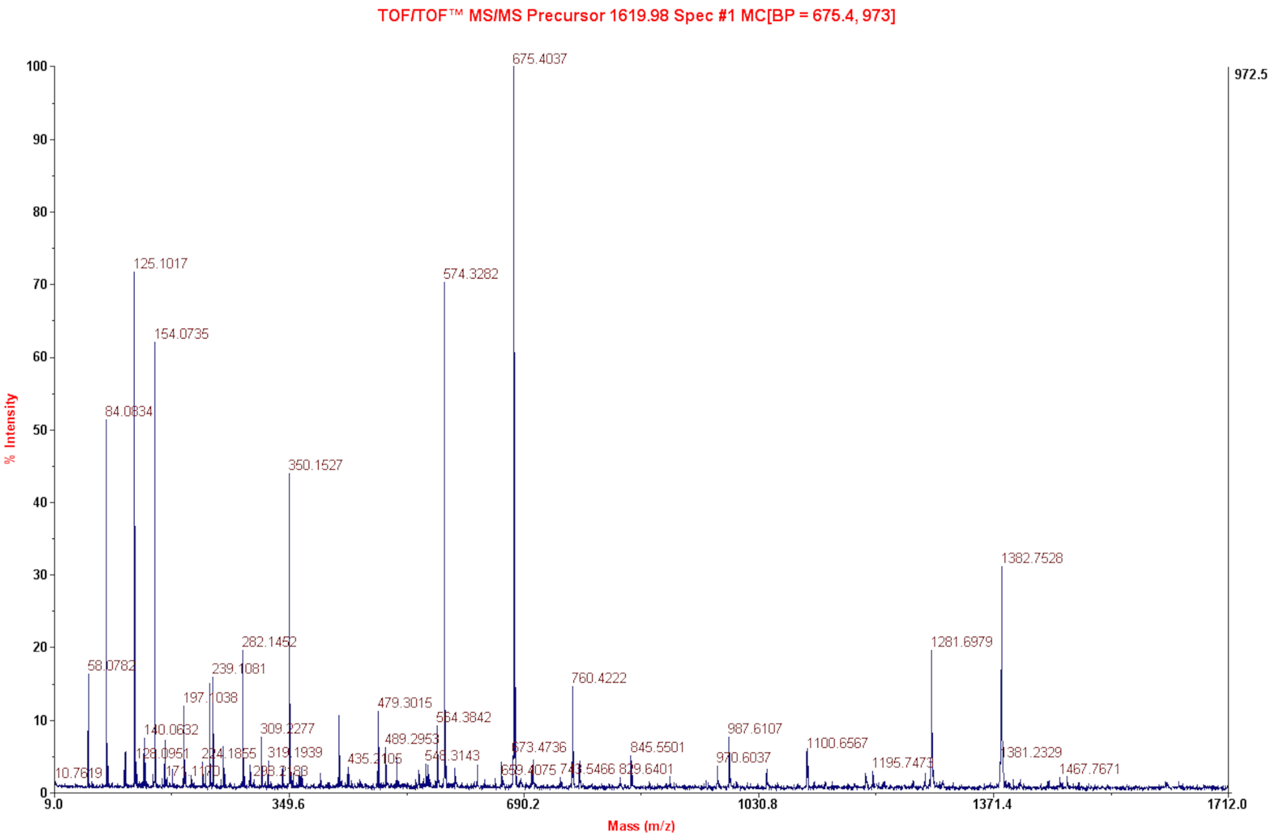


Figure S45. MS/MS spectrum of **1d**.

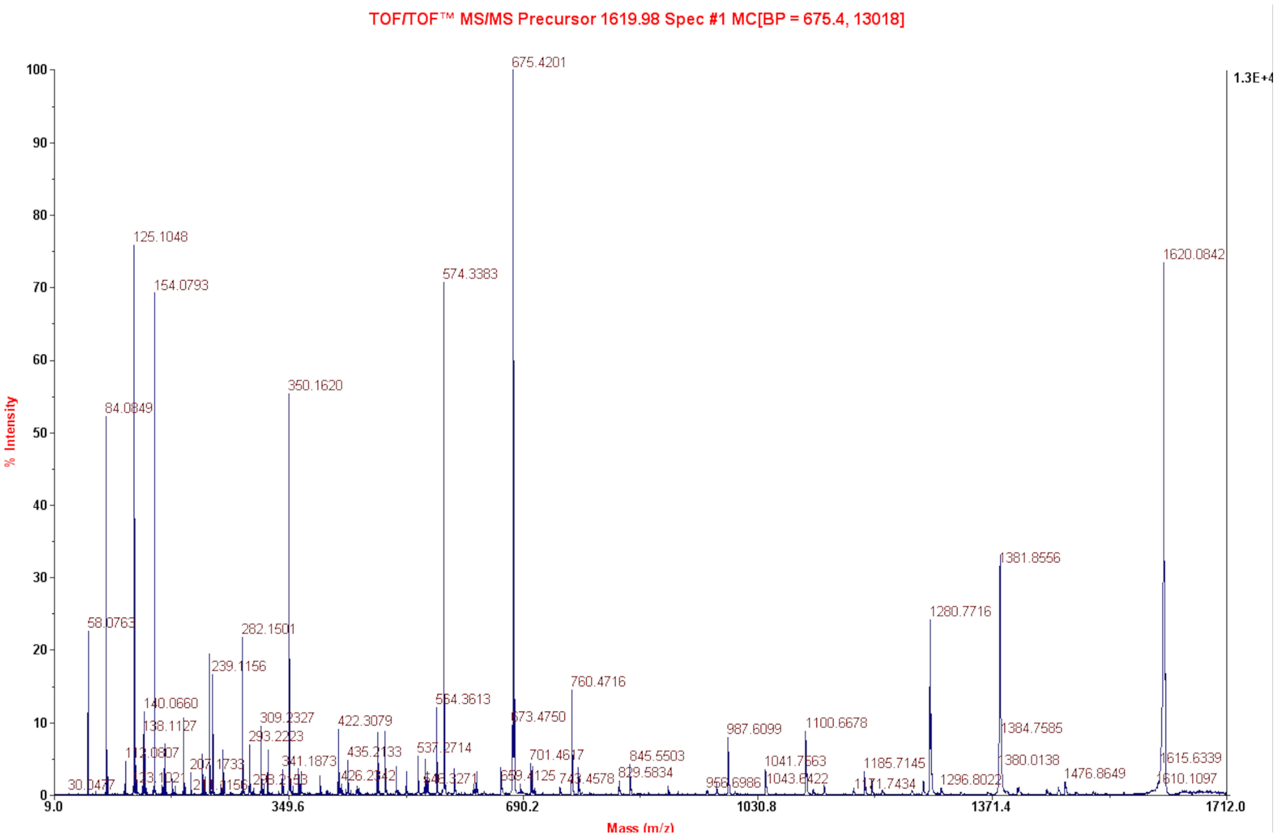


Figure S46. MS/MS spectrum of **1e**.

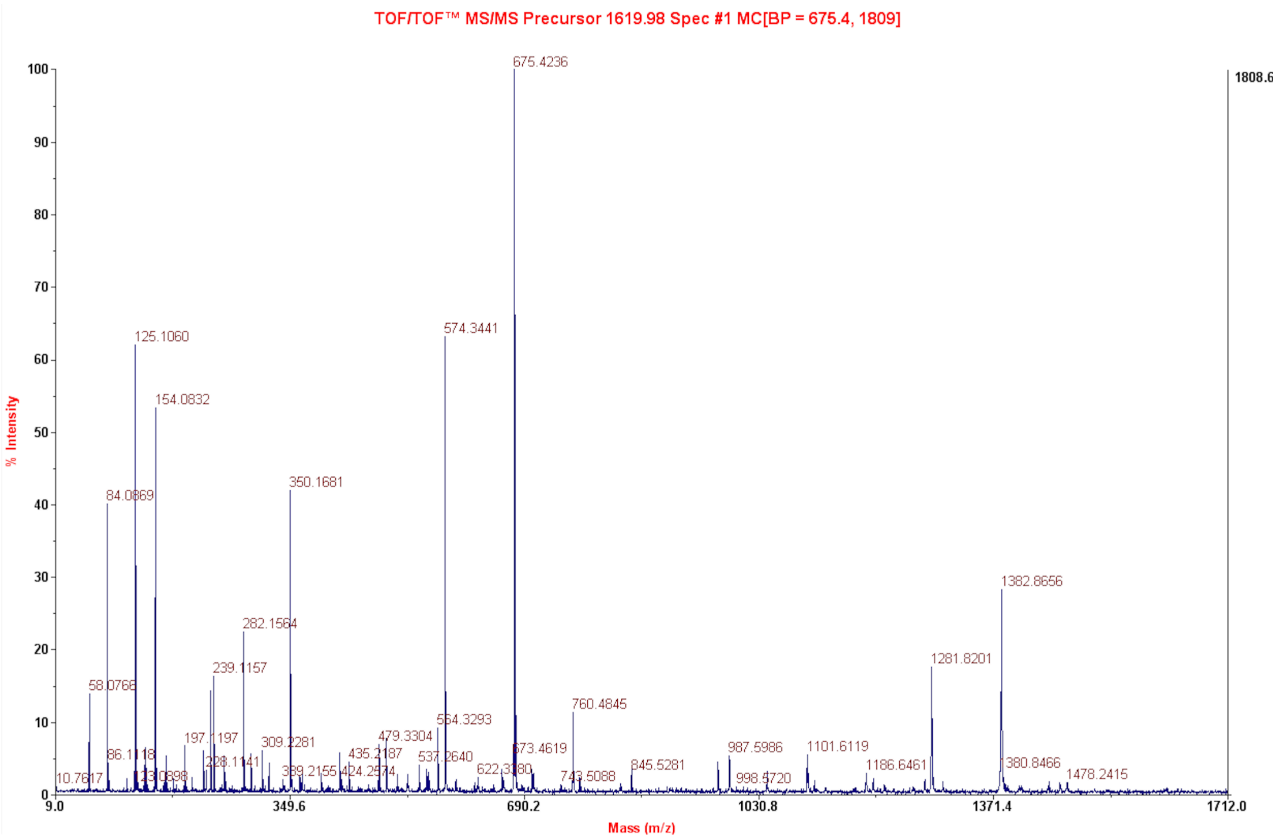


Figure S47. MS/MS spectrum of **1f**.

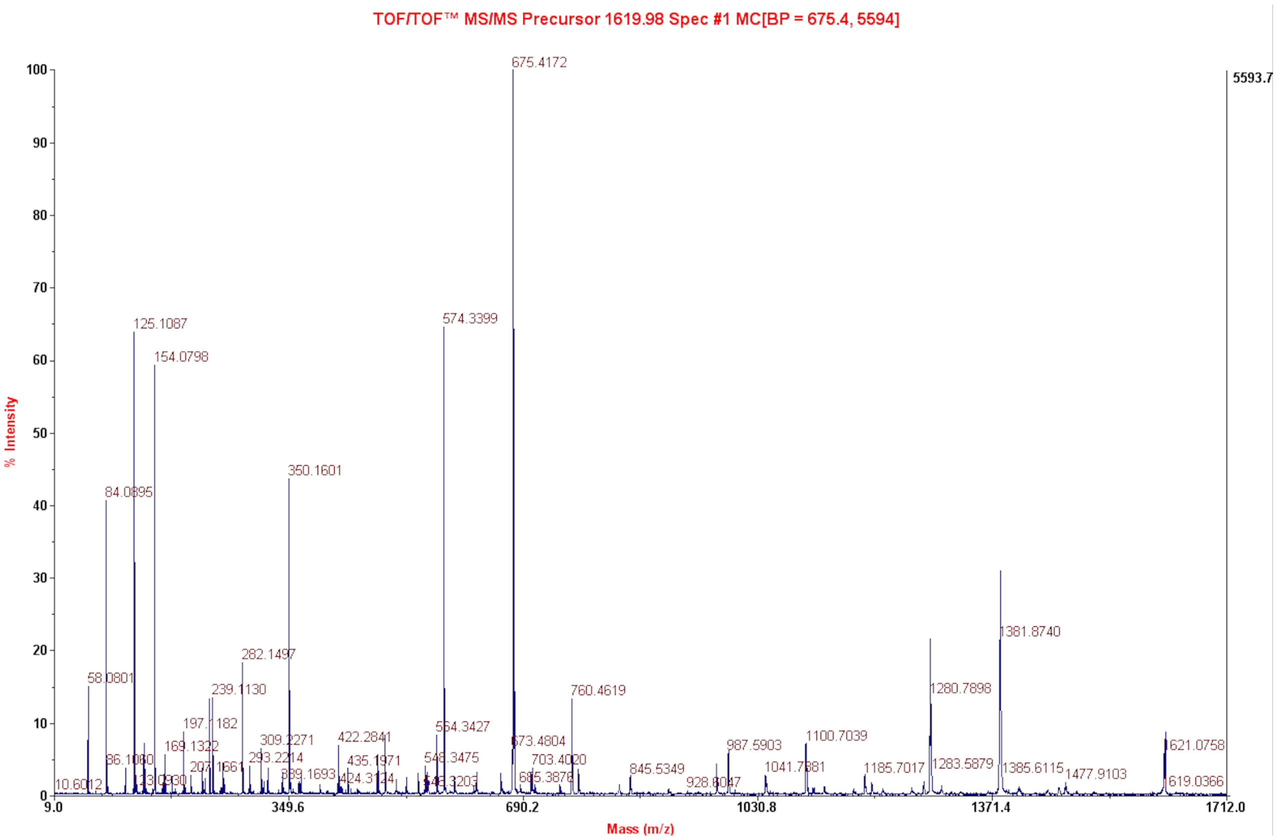


Figure S48. MS/MS spectrum of **1g**.

HPLC and UHPLC Charts

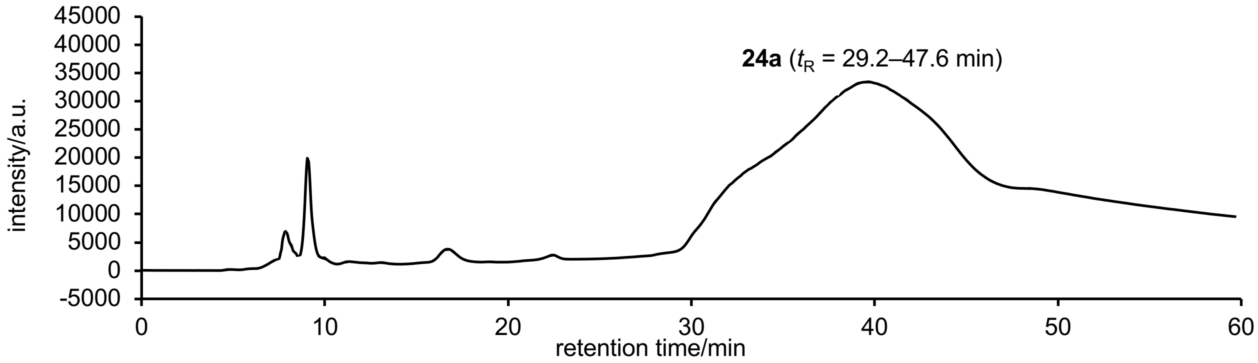


Figure S49. HPLC chart for purification of **24a**. Column: Inertsil C8-3 10 × 250 mm, eluent A: *i*-PrOH, eluent B: H_2O, linear gradient A/B = 70/30 to 100/0 over 30 min, flow rate: 1.50 mL/min, detection: UV 220 nm, temperature: 40 °C.

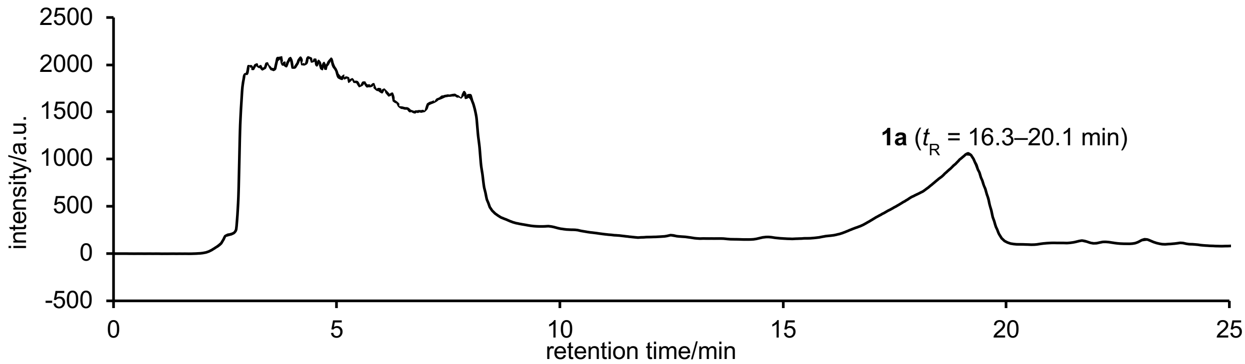


Figure S50. HPLC chart for purification of **1a**. Column: Inertsil C8-3 4.6 × 150 mm, eluent A: *i*-PrOH, eluent B: H_2O, linear gradient A/B = 90/10 to 100/0 over 60 min, flow rate: 0.500 mL/min, detection: UV 220 nm, temperature: 40 °C.

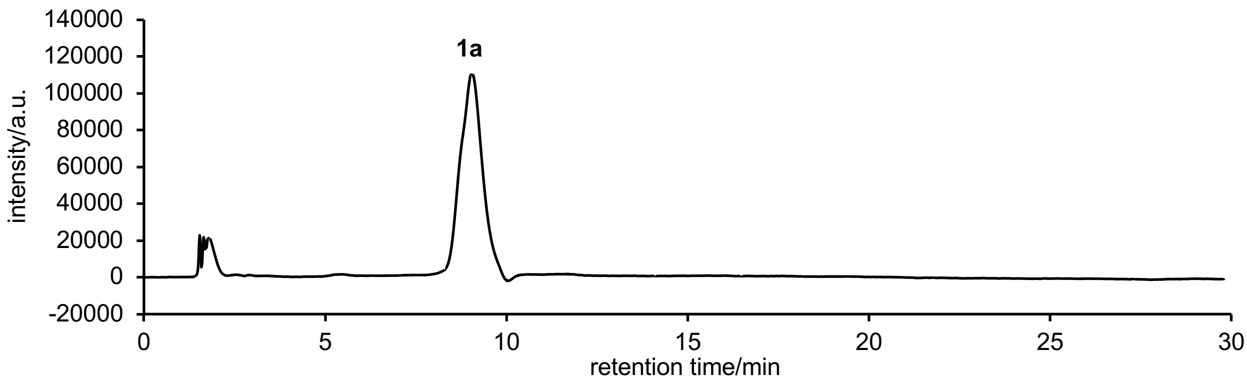


Figure S51. UHPLC chart for purified **1a**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

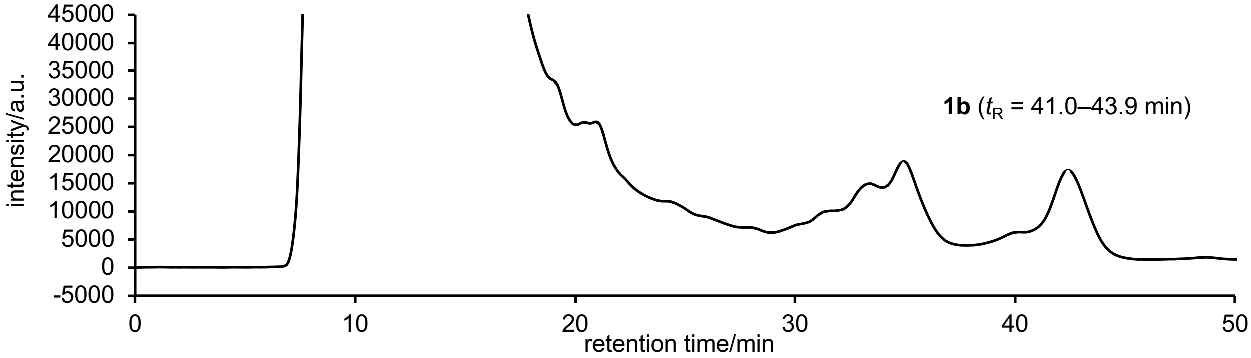


Figure S52. HPLC chart for purification of **1b**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

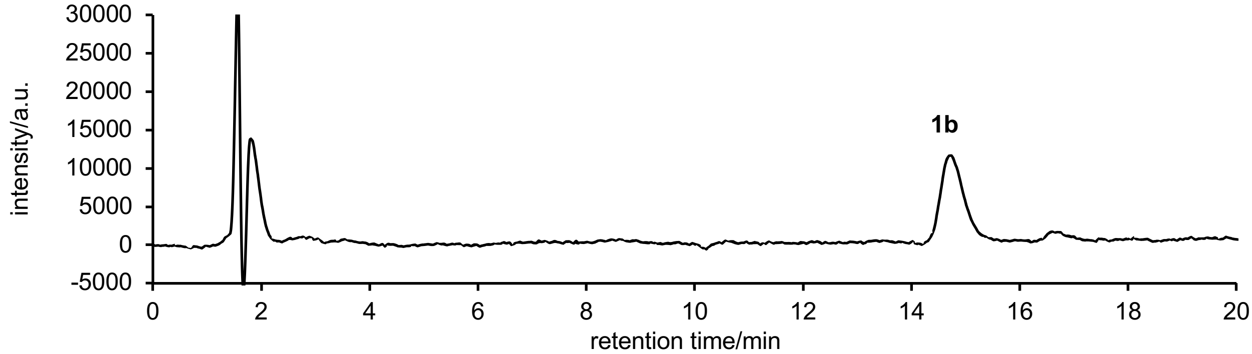


Figure S53. UHPLC chart for purified **1b**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

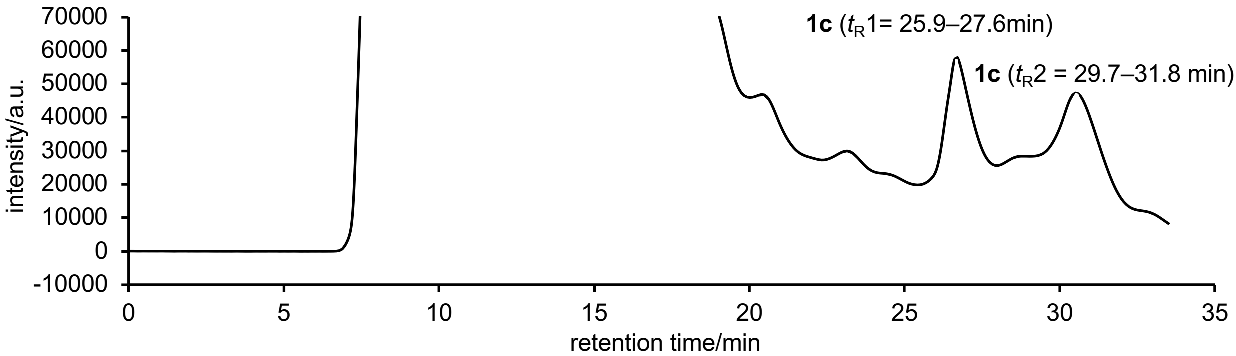


Figure S54. HPLC chart for purification of **1c**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

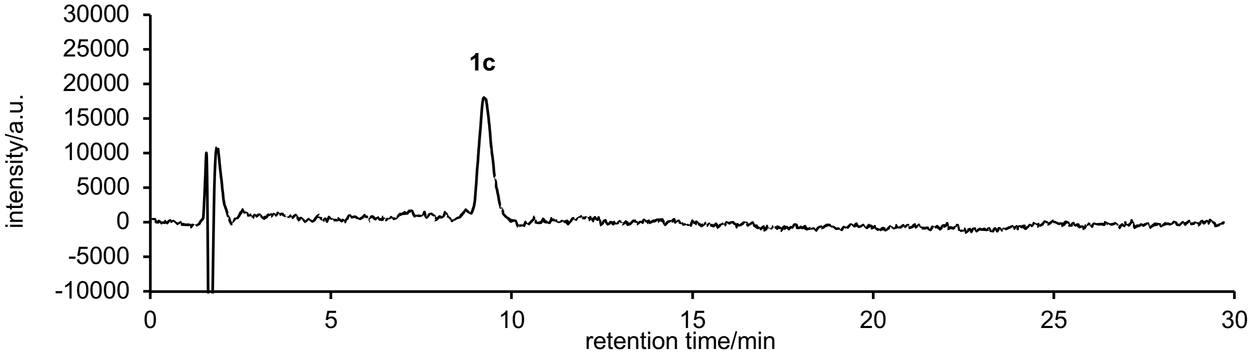


Figure S55. UHPLC chart for purified **1c**. The combined fractions t_R1 and t_R2 in Figure S54 gave a single peak, indicating that the two fractions contained pure **1c**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

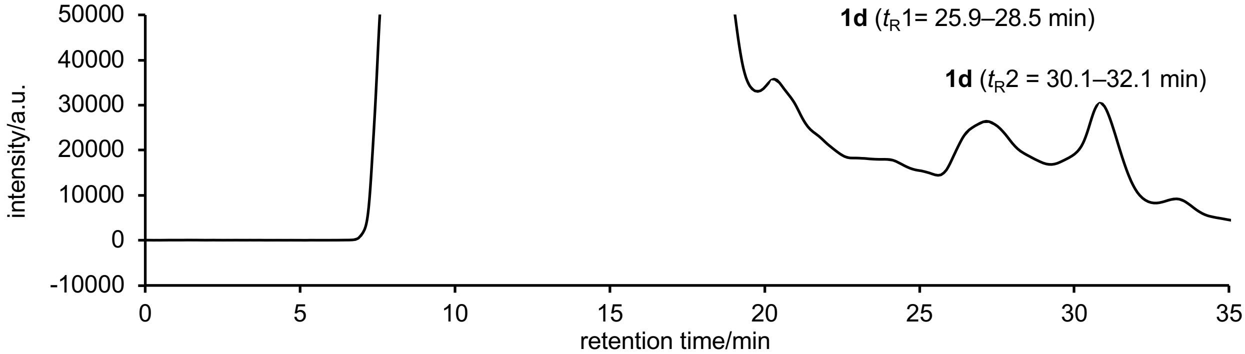


Figure S56. HPLC chart for purification of **1d**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

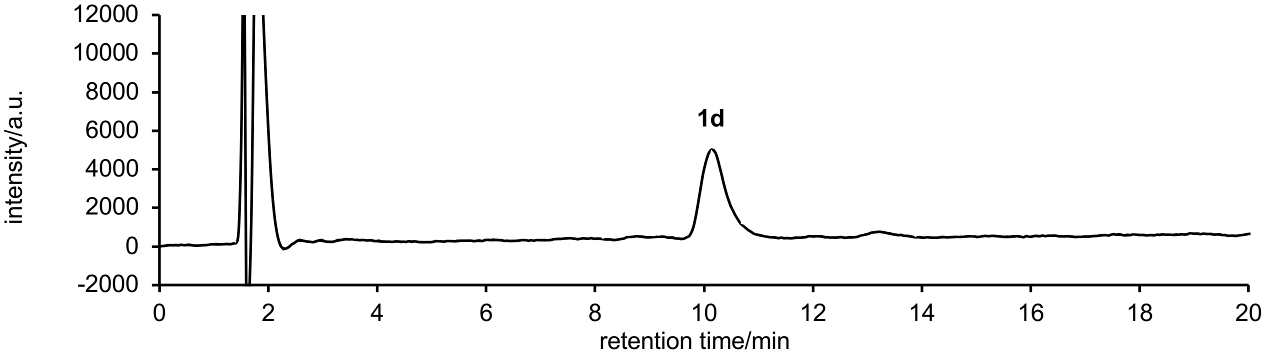


Figure S57. UHPLC chart for purified **1d**. The combined fractions t_R1 and t_R2 in Figure S56 gave a single peak, indicating that the two fractions contained pure **1d**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

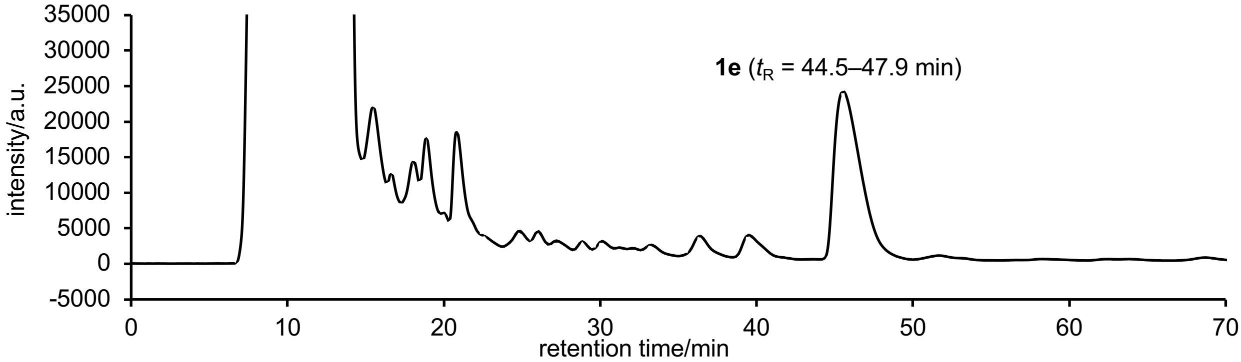


Figure S58. HPLC chart for purification of **1e**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

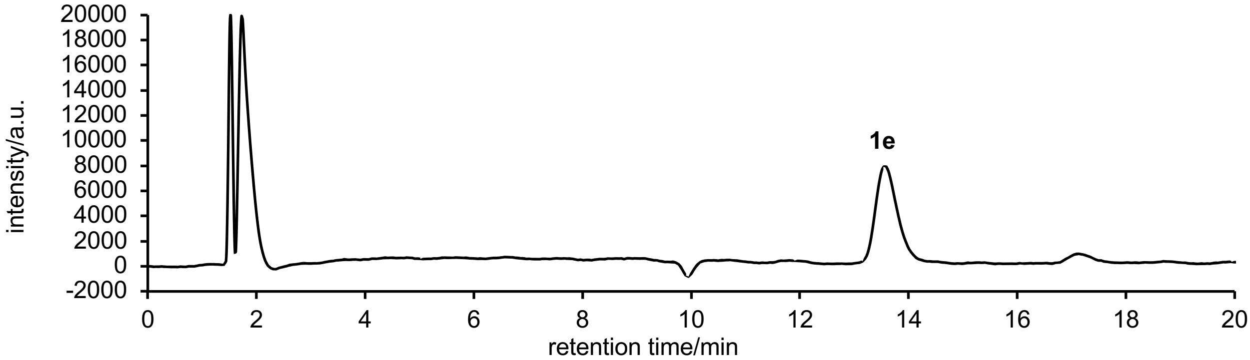


Figure S59. UHPLC chart for purified **1e**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

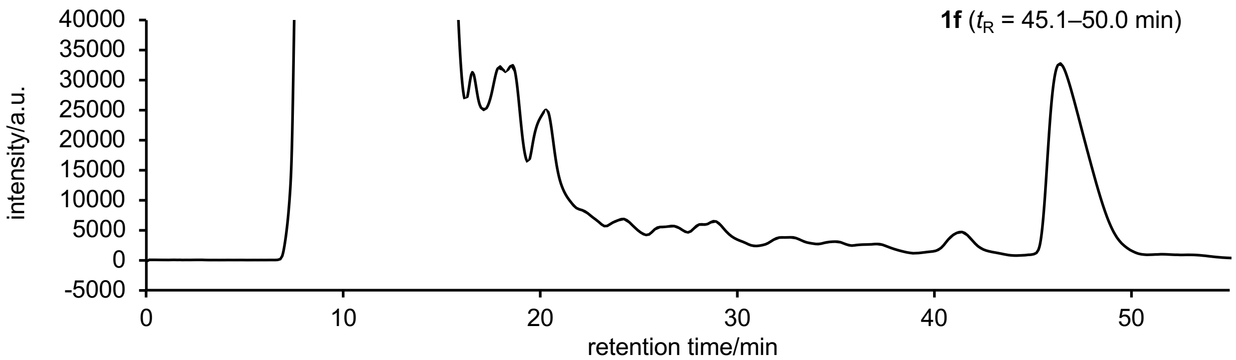


Figure S60. HPLC chart for purification of **1f**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

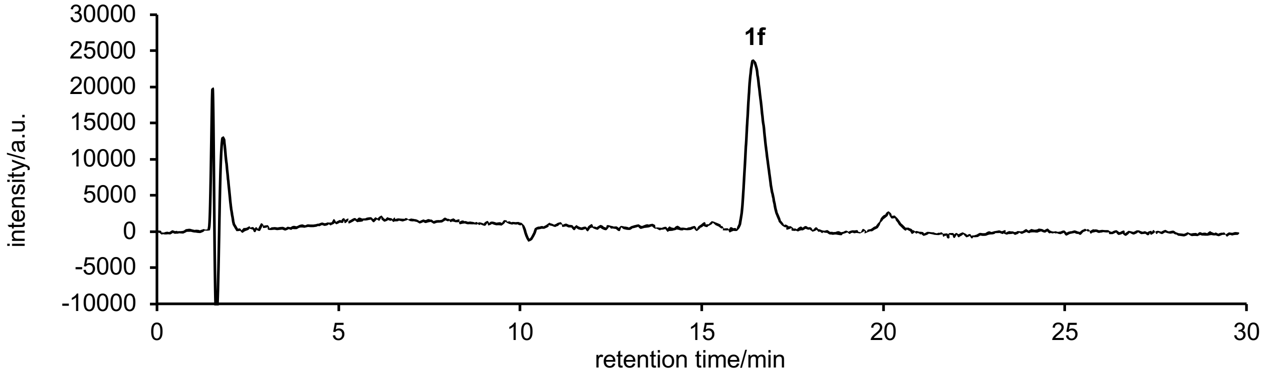


Figure S61. UHPLC chart for purified **1f**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

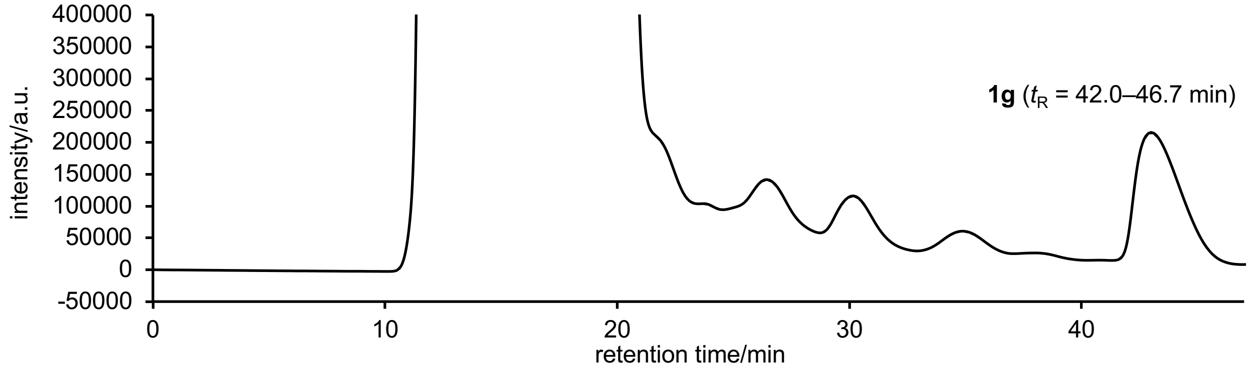


Figure S62. HPLC chart for purification of **1g**. Column: Inertsil ODS-4 10 × 250 mm, eluent A: *i*-PrOH/MeCN = 1/2 + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 60/40, flow rate: 2.50 mL/min, detection: UV 220 nm, temperature: 40 ºC.

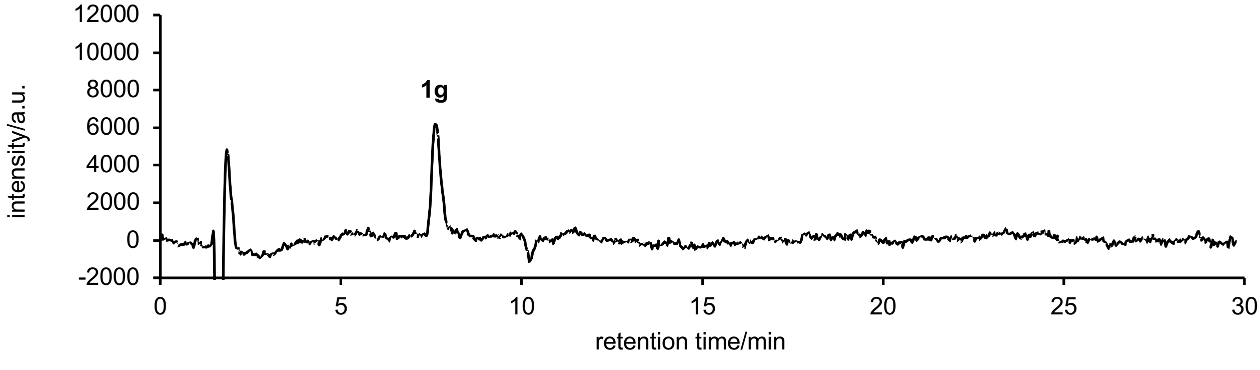


Figure S63. UHPLC chart for purified **1g**. Column: Accucore C18 2.1 × 150 mm, eluent A: *i*-PrOH + 0.05% TFA, eluent B: H_2O + 0.05% TFA, A/B = 30/70, flow rate: 0.200 mL/min, detection: photodiode array detector 200–648 nm (UV chromatogram: 220 nm), temperature: 40 ºC.

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